JC02 Rec'd PCT/PTO 25 MAR 2002

Attorney Docket No.: NIH-05111

10/089009

TRANSMITTAL LETTER TO THE UNITED STATES ELECTED OFFICE (EO/US) (ENTRY INTO U.S. NATIONAL PHASE UNDER CHAPTER II PCT)

International Appln. No.:	International Filing Date:	Priority Date Claimed:
PCT/US00/25963	21 September 2000 (21.09.00)	24 September 1999 (24.09.99)

Title of Invention: **INTERLEUKIN-2 RECEPTOR ASSOCIATED POLYPEPTIDES**

Applicant: THE UNITED STATES OF AMERICA, as represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES

CERTIFICATION UNDER 37 C.F.R. § 1.10

I hereby certify that this New Application Transmittal and the documents referred to as enclosed therein are being deposited with the U.S. Postal Service on this date **March 25, 2002** in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number **EV 008 739 588 US** addressed to: **Box PCT, ATTN. EO/US**, Assistant Commissioner For Patents, Washington, D.C. 20231.

James R. Davenport

BOX PCT
Assistant Commissioner For Patents
Washington, D.C. 20231
ATTN: EO/US

1. a. X **This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.**
   b. __ **This is a SECOND or SUBSEQUENT submission of items concering a filing under 35 U.S.C. 371.**

2. **Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 U.S.C. 371:**
   a. X This express request to immediately begin national examination procedures (35 U.S.C. 371(f).
   b. X The U.S. National Fee (35 U.S.C. 371(c)(1) and other fees (37 CFR 1.492) as indicated below:

3. **Fees**

Regular application

CLAIMS AS FILED

Number Filed	Number Extra	Rate	Calculations
Total Claims (37 C.F.R. § 1.16(c))	21 - 20 =	1 x $18.00 =	$18.00
Independent Claims (37 C.F.R. § 1.16(b))	7 - 3 =	4 x $84.00 =	$336.00
Multiple Dependent Claim(s), if any (37 C.F.R. § 1.16(d))		+ $280.00 =	$0.00
		Filing Fee Calculation	$354.00

X U.S. PTO WAS INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY
Where an international preliminary examination fee as set forth in § 1.482 has been paid on the international application to the U.S. PTO **(however; an IPER had not issued as of 25 March 2002)**.

__ and the international preliminary examination report states that the criteria of novelty, inventive step (non-obviousness) and industrial activity, as defined in PCT Article 33(1) to (4) have been satisfied for all the claims presented in the application entering the national stage (37 CFR 1.492(a)(4) 100.00 _____

__ and the above requirements are not met (37 CFR 1.492(a)(1) 690.00 690.00

U.S. PTO WAS NOT INTERNATIONAL PRELIMINARY EXAMINATION AUTHORITY

Where no international preliminary examination fee as set forth in § 1.482 has been paid to the U.S. PTO, and payment of an international search fee as set forth in § 1.445(a)(2) to the U.S. PTO:

__	has been paid (37 CFR 1.492(a)(2))	710.00	_____
__	has not been paid (37CFR 1.492(a)(3)	1000.00	_____
__	where a search report on the international application has been prepared by the European Patent Office or the Japanese Patent Office (37 CFR 1.492(a)(5)	860.00	_____

Total National Fee	**1,044.00**
Small Entity - Reduction by 1/2, if applicable	_____
Total Fees Enclosed	**1,044.00**

X Check in the amount of **$1,044.00** to cover the above fees is enclosed.

4. **A copy of the International application as published (35 U.S.C. 371(c)(2)):**
   - a. X is transmitted herewith.
   - b. __ is not required, as the application was filed with the United States Receiving Office.
   - c. __ has been transmitted
     - i. __ by the International Bureau.
       Date of mailing of the application (from form PCT/1B/308): ___.
     - ii. __ by applicant on (date) _____.

5. **A Translation of the International application into the English language (35 U.S.C. 371(c)(2)):**
   - a. __ is transmitted herewith.
   - b. X is not required as the application was filed in English.
   - c. __ was previously transmitted by applicant on (date) _____.
   - d. __ will follow.

6. **Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. 371(c)(3)):**
   - a. __ are transmitted herewith (as a part of the International Examination Report)
   - b. __ have been transmitted
     - i. __ by the International Bureau.
       Date of mailing of the amendment (from form PCT/1B/308): _____
     - ii. __ by applicant on (date) _____.
   - c. X have not been transmitted as
     - i. X applicant chose not to make amendments under PCT Article 19. Date of mailing of Search Report (from form PCT/ISA/210.): 06 August 2001 (06.08.01).
     - ii. __ the time limit for the submission of amendments has not yet expired. The amendments or a statement that amendments have not been made will be transmitted before the expiration of the time limit under PCT Rule 46.1.

7. **A translation of the amendments to the claims under PCT Article 19 (38 U.S.C. 371(c)(3)):**
   - a. __ is transmitted herewith.
   - b. __ is not required as the amendments were made in the English language.
   - c. X has not been transmitted for reasons indicated at point 6c above.

8. **A copy of the international examination report (PCT/IPEA/409)**
   - __ is transmitted herewith.
   - X is not required as the application was filed with the United States Receiving Office.

9. **Annex(es) to the international preliminary examination report**
   - a. __ is/are transmitted herewith.
   - b. X is/are not required as the application was filed with the United States Receiving Office.

10. **A translation of the annexes to the international preliminary examination report** JC13 Rec'd PCT/PTO 25 MAR 2002
    a. __ is transmitted herewith.
    b. X is not required as the annexes are in the English language.

11. **An oath or declaration of the inventor (35 U.S.C. 371(c)(4)) complying with 35 U.S.C. 115**
    a. __ was previously submitted by applicant on (date) _____.
    b. X is submitted herewith, and such **executed** oath or declaration
       i. __ is attached to the application.
       ii. __ identifies the application and any amendments under PCT Article 19 that were transmitted as stated in points 3b or 3c and 5b; and states thay they were reviewed by the inventor as required by 37 CFR 1.70.
       iii. X will follow.

II. **Other document(s) or information included:**

12. **An International Search Report (PCT/ISA/210) or Declaration under PCT Article 17(2)(a):**
    a. __ is transmitted herewith.
    b. __ has been transmitted by the International Bureau. Date of mailing (from form PCT/IB/308): _____
    c. __ is not required, as the application was searched by the United States International Searching Authority.
    d. X will be transmitted promptly upon request.
    e. __ has been submitted by applicant on (date) _____

13. **An Information Disclosure Statement under 37 CFR 1.97 and 1.98:**
    a. __ is transmitted herewith.
       Also transmitted herewith is/are:
       __ Form PTO-1449.
       __ Copies of citations listed.
    b. X will be transmitted within THREE MONTHS of the date of submission of requirements under 35 U.S.C. 371(c).
    c. __ was previously submitted by applicant on (date) _____.

14. **An Executed Assignment document:**
    a. X will follow.
    b. __ is transmitted herewith for recording.
       A separate __ 'COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or __ FORM PTO 1595 is also attached.

15. **Additional Documents**
    a. __ Copy of Request (PCT/RO/101)
    b. X International Publication No. WO 01/23428.
       i. X Specification, claims and drawing
       ii. __ Front page only.
    c. __ Preliminary amendment (37 CFR ξ 1.121)
    d. __ Other
       Assignment (unexecuted)

16. **The above checked items are being transmitted**
    a. X before 30 months from any claimed priority date.
    b. __ after 30 months.

17. Certain requirements under 35 U.S.C. 371 were previously submitted by the applicant on _____, namely:

18. **Authorization To Charge Additional Fees and Credit Overpayment**

X The Commissioner is hereby authorized to charge payment of any additional fees that may be required by this paper including, without limitation, filing fees, fees for presentation of extra claims, and surcharge for filing late declarations to Deposit Account No.: **08-1290**.
An originally executed duplicate of this transmittal is enclosed for this purpose.

19. **Return Receipt Postcard**

X Enclosed.

Dated: March 25, 2002

HOM, Judith S.H.
Registration No.: 44,772

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PATENT
Attorney Docket No. 218811
DHHS Ref: E-025-99

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Goldman et al.

Application No. 10/089,009

Filed: March 25, 2002

For: INTERLEUKIN-2 RECEPTOR ASSOCIATED POLYPEPTIDES

Art Unit: Unassigned

Examiner: Unassigned

PRELIMINARY AMENDMENT

Commissioner for Patents
Box Sequence
P.O. Box 2327
Arlington, VA 22202

Dear Sir:

Prior to the examination of the above-identified patent application, please enter the following amendments and consider the following remarks.

AMENDMENTS

IN THE SPECIFICATION:

Please amend the specification at page 17, lines 3-14, to read as follows:

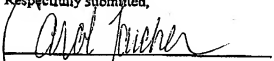
As used herein, the terms "complementary" or "complementarity" are used in reference to "polynucleotides" and "oligonucleotides" (which are interchangeable terms that refer to a sequence of nucleotides) related by the base-pairing rules. For example, the sequence "5'-CAGT-3'" (SEQ ID NO.: 1) is complementary to the sequence "5'-ACTG-3'" (SEQ ID NO.: 2). Complementarity can be "partial" or "total." "Partial" complementarity is where one or more nucleic acid bases is not matched according to the base pairing rules. "Total" or "complete" complementarity between nucleic acids is where each and every nucleic acid base is matched with another base under the base

pairing rules. The degree of complementarity between nucleic acid strands may have significant effects on the efficiency and strength of hybridization between nucleic acid strands. This may be of particular importance in amplification reactions, as well as detection methods which depend upon binding between nucleic acids.

REMARKS

The specification has been amended at page 17, lines 3-14, to add sequence identification numbers. A separate document setting forth the precise changes to the specification is enclosed. The application is considered to be in good and proper form for allowance, and the Examiner is respectfully requested to pass this application to issue. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,



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Date: January 16, 2003

In re Appln. of Goldman et al.
Application No. 10/089,009

CERTIFICATE OF EXPRESS MAILING

I hereby certify that this PRELIMINARY AMENDMENT (along with any documents referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below in an express mail envelope, mailing label number EL190837027US, addressed to: Commissioner for Patents, Box Sequence, P.O. Box 2327, Arlington, VA 22202

Date: 1/16/03 _____ _____

PATENT
Attorney Docket No. 218811
DHHS Ref: E-025-99

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Goldman et al.

Application No. 10/089,009

Filed: March 25, 2002

For: INTERLEUKIN-2 RECEPTOR ASSOCIATED POLYPEPTIDES

Art Unit: Unassigned

Examiner: Unassigned

AMENDMENTS TO SPECIFICATION MADE VIA PRELIMINARY AMENDMENT

(insertions indicated by underlining; deletions indicated by brackets)

Amendment to the specification at page 17, lines 3-14:

As used herein, the terms "complementary" or "complementarity" are used in reference to "polynucleotides" and "oligonucleotides" (which are interchangeable terms that refer to a sequence of nucleotides) related by the base-pairing rules. For example, the sequence "5'-CAGT-3'[,]" (SEQ ID NO.: 1) is complementary to the sequence "5'-ACTG-3'[.]" (SEQ ID NO.: 2). Complementarity can be "partial" or "total." "Partial" complementarity is where one or more nucleic acid bases is not matched according to the base pairing rules. "Total" or "complete" complementarity between nucleic acids is where each and every nucleic acid base is matched with another base under the base pairing rules. The degree of complementarity between nucleic acid strands may have significant effects on the efficiency and strength of hybridization between nucleic acid strands. This may be of particular importance in amplification reactions, as well as detection methods which depend upon binding between nucleic acids.

<110> Goldman, Carolyn K
Goldman, Neil D
Waldmann, Thomas A

<120> INTERLEUKIN-2 RECEPTOR ASSOCIATED POLYPEPTIDES

<130> 218811

<140> 10/089,009
<141> 2002-03-25

<150> PCT/US00/25963
<151> 2000-09-21

<150> 60/155,968
<151> 1999-09-24

<160> 2

<170> PatentIn version 3.1

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actg 4

WO 01/23428 PCT/US00/25963

INTERLEUKIN-2 RECEPTOR ASSOCIATED POLYPEPTIDES

FIELD OF THE INVENTION

The present invention relates to two polypeptides which are associated with the interleukin-2 receptor (IL-2R) in the plasma membrane of cells expressing the IL-2R. These two polypeptides, designated interleukin-2 receptor associated polypeptides, have molecular weights of about 32,000 to 34,000 daltons and 26,000 to 28,000 daltons, respectively, and are reactive with the monoclonal antibody 5F7. The invention therefore relates to antibodies specific for these polypeptides and to the use of these antibodies for the detection of these polypeptides and for their isolation and purification from biological samples.

BACKGROUND OF THE INVENTION

Immune responses are regulated by a series of proteins termed cytokines that exhibit a high degree of redundancy and pleiotropy in controlling a wide range of functions in various cell types. The redundancy is explained in part since even though each cytokine has its own private receptor, it usually also shares common receptor subunits with other cytokines. An example of the phenomenon is the interleukin 2 (IL-2)/IL-2 receptor (IL-2R) system which has long been recognized to play a critical role in the proliferation, differentiation, survival and functional activities of lymphocytes (Waldmann TA, *et al.*, "The interleukin-2 receptor: a target for monoclonal antibody treatment of human T-cell lymphotrophic virus I-induced adult T-cell leukemia" *Blood* 82:1701-1712, 1993; Waldmann TA, *et al.*, "Radioimmunotherapy of interleukin-2R alpha-expressing adult T-cell leukemia with Yttrium-90-labeled anti-Tac" *Blood* 86:4063-4075, 1995; Waldmann TA, *et al.* "Genetically engineered monoclonal antibodies armed with radionuclides" *Intern Rev Immunol* 16:205-226, 1998). The IL-2R system comprises three IL-2 binding proteins, a 55 kDa IL-2Rα subunit, a 70/75 kDa IL-2Rβ subunit, and a 64 kDa IL-2Rγ subunit, which in various combinations, form receptors having high, low and intermediate affinity receptors for IL-2 (Tsudo M, *et al.* "Demonstration of a non-Tac peptide that binds interleukin 2: a potential participant in a multichain interleukin 2 receptor complex" *Proc Natl Acad Sci USA* 83:9694-9698, 1986; Tsudo M, *et al.* "The p75 peptide is the receptor for interleukin 2 expressed on large granular lymphocytes and is responsible for the interleukin 2 activation of these cells" *Proc Natl Acad Sci USA* 84:5394-5398, 1987;

Waldmann TA., "Multichain interleukin-2 receptor: a target for immunotherapy in lymphoma" *J Natl Cancer Inst* 81:914-923, 1989; Waldmann TA and O'Shea J, "The use of antibodies against the IL-2 receptor in transplantation" *Curr Opin Immunol* 10:507-512, 1998; Waldmann TA, *et al* "Genetically engineered monoclonal antibodies armed with radionuclides" *Intern Rev Immunol* 16:205-226, 1998). In brief, the high affinity receptor consists of all three subunits, the intermediate affinity receptor consists of the β and γ subunits and the low affinity receptor consists only of the α subunit. IL-2Rα is specific for IL-2, but IL-2Rγ (now termed common γ (γc)), is also required for the actions of IL-4, IL-7, IL-9 and IL-15. IL-2Rβ is also utilized by IL-15. IL-2 and IL-15 share a number of biological activities (Waldmann TA., "Multichain interleukin-2 receptor: a target for immunotherapy in lymphoma" *J Natl Cancer Inst* 81:914-923, 1989; Waldmann TA, et al., Blood 1993, 82: 1701-12; Waldmann TA, O'Shea J. Curr Opin Immunol 1998, 10:507-12; Waldmann TA, *et al.* "Genetically engineered monoclonal antibodies armed with radionuclides" *Intern Rev Immunol* 16:205-226, 1998).

In addition to its shared functions with other cytokines such as IL-15, IL-2 appears to have a unique function not shared with other cytokines. In particular, based on experiments in which mice were made deficient in IL-2, IL-2Rα or IL-2Rβ by gene targeting, the mice manifested only a modest immune deficiency but developed lymphocytosis, hyper-gammaglobulinemia, and hemolytic anemia. It has been suggested that the mechanism underlying the increased lymphoproliferation and autoimmune disease in mice with deficiencies in IL-2, IL-2Rα or IL-2Rβ is via the loss of an essential contribution to activation induced cell death (AICD), a phenomenon involved in the maintenance of peripheral self-tolerance normally provided by IL-2 and its receptor system (Waldmann TA, *et al.* "Genetically engineered monoclonal antibodies armed with radionuclides" *Intern Rev Immunol* 16:205-226, 1998). In brief, following persistent T cell receptor stimulation, IL-2 is produced and interacts with IL-2R on the stimulated T cell. If these IL-2-stimulated cycling cells are reexposed to the original antigen, they express the fas ligand or TNFα (tumor necrosis factor α), effector molecules required for the apoptotic cell death of mature peripheral T cells. However, as IL-2Rβ and IL-2Rγ are shared with IL-15, a cytokine that is not involved in AICD, these receptor subunits do not appear to provide a sufficient receptor/signaling pathway for this IL-2 specific response. Furthermore, IL-2Rα, the private receptor for IL-2, is not apparently directly involved in IL-2-mediated signaling, since it contains only 13 cytoplasmic amino acids, none of which

are a tyrosine (Waldmann TA., "Multichain interleukin-2 receptor: a target for immunotherapy in lymphoma" *J Natl Cancer Inst* 81:914-923, 1989; Waldmann TA, *et al.* "Genetically engineered monoclonal antibodies armed with radionuclides" *Intern Rev Immunol* 16:205-226, 1998). Therefore, it appears that the IL-2 specific AICD phenomenon might be mediated by an as yet undefined molecule that associates with IL-2Rα.

SUMMARY OF THE INVENTION

The present invention relates to the identification of two polypeptides which associate with the IL-2R, and are immunologically recognized by the monoclonal antibody 5F7 produced from the murine hybridoma having American Type Culture Collection (ATCC) deposit number PTA-82. These polypeptides, designated interleukin-2 receptor associated polypeptides or ILRAPs, have molecular weights of about 32,000 to 34,000 daltons and 26,000 to 28,000 daltons, respectively.

The invention also provides antibodies specific for the polypeptides of the invention. In alternative embodiments, the present invention provides various methods for the use of these antibodies, including but not limited to the isolation and purification of ILRAPs, as well as for the detection of ILRAPs in biological samples.

The present invention provides compositions comprising at least one IL-2 receptor associated polypeptide (ILRAP), wherein the ILRAP is reactive with the monoclonal antibody produced by the hybridoma having ATCC deposit No. PTA-82. The present invention further provides compositions comprising at least one ILRAP, wherein the ILRAP has a molecular weight of about 32,000 to 34,000 daltons and/or a molecular weight of about 26,000 to 28,000 daltons. The present invention further provides compositions comprising ILRAP, wherein the ILRAP is expressed in cells, including but not limited to Kit-225 cells and HuT 102 cells. The present invention further provides compositions comprising at least one ILRAP, wherein the ILRAP associates with the IL-2Rα subunit.

The present invention also provides antibodies directed against ILRAP. For preparation of monoclonal antibodies directed toward ILRAP, any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used (*See, e.g.,* Harlow and Lane, *Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press*, Cold Spring Harbor, NY). These include but are not limited to the

hybridoma technique originally developed by Köhler and Milstein (Köhler and Milstein, Nature 256:495-497 [1975]), as well as the trioma technique, the human B-cell hybridoma technique (*See e.g.*, Kozbor *et al.* Immunol. Today 4:72 [1983]), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole *et al., in Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 [1985]).

In additional embodiments of the invention, monoclonal antibodies can be produced in germ-free animals (*See e.g.*, PCT/US90/02545). According to the invention, human antibodies may be used and can be obtained by using human hybridomas (Cote *et al.*, Proc. Natl. Acad. Sci. U.S.A.80:2026-2030 [1983]) or by transforming human B cells with EBV virus in vitro (Cole *et al.*, *in Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, pp. 77-96 [1985]). In addition, invention, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778; herein incorporated by reference) can be adapted to produce ILRAP-specific single chain antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries (Huse *et al.*, Science 246:1275-1281 [1989]) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for ILRAP.

Antibody fragments which contain the idiotype (antigen binding region) of the antibody molecule can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')2 fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the F(ab')2 fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art (*e.g.*, radioimmunoassay, ELISA [enzyme-linked immunosorbent assay], "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in situ* immunoassays [using colloidal gold, enzyme or radioisotope labels, for example], Western Blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays, etc.), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc.

In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further

embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention. (As is well known in the art, the immunogenic peptide should be provided free of the carrier molecule used in any immunization protocol. For example, if the peptide was conjugated to KLH, it may be conjugated to BSA, or used directly, in a screening assay).

The foregoing antibodies can be used in methods known in the art relating to the localization and structure of ILRAP (*e.g.*, for Western blotting), measuring levels thereof in appropriate biological samples, etc. The antibodies can be used to detect ILRAP in a biological sample from an individual. The biological sample can be a biological fluid, such as but not limited to, blood, serum, plasma, interstitial fluid, urine, cerebrospinal fluid, and the like, containing cells. In particular, ILRAP can be detected from cellular sources.

The biological samples can then be tested directly for the presence of ILRAP using an appropriate strategy (*e.g.*, ELISA or radioimmunoassay) and format (*e.g.*, microwells, dipstick [*e.g.*, as described in International Patent Publication WO 93/03367], etc.). Alternatively, proteins in the sample can be size separated (*e.g.*, by polyacrylamide gel electrophoresis (PAGE), in the presence or not of sodium dodecyl sulfate (SDS), and the presence of ILRAP detected by immunoblotting (Western blotting)).

The foregoing explanations of particular assay systems are presented herein for purposes of illustration only, in fulfillment of the duty to present an enabling disclosure of the invention. It is to be understood that the present invention contemplates a variety of immunochemical assay protocols within its spirit and scope.

In particularly preferred embodiments, the present invention provides compositions comprising the antibody produced by the hybridoma having ATCC deposit No. PTA-82. In some particularly preferred embodiments, the antibody is monoclonal. In further particularly preferred embodiments, the antibody is of the IgM class. In still other embodiments, the antibody is a polyclonal antibody that is capable of recognizing at least one ILRAP.

The present invention also provides methods for purifying at least one IL-2 receptor associated polypeptide (ILRAP), comprising the step of contacting IL-2 receptor expressing cells with an anti-ILRAP antibody under conditions where the antibody forms a complex with the ILRAP. In alternative embodiments, the present invention provides for solubilizing the IL-2 receptor/ILRAP expressing cells before contacting them with the anti-

ILRAP antibody. The present invention further provides methods wherein the anti-ILRAP antibody is present on an immunoaffinity column. In some embodiments, the present invention provides methods wherein the ILRAP bound to the anti-ILRAP antibody is eluted from the immunoaffinity column. The present invention further provides methods wherein the IL-2 receptor expressing cells are selected from the group consisting of cells lines expressing the IL-2R α and cell lines expressing IL-2R β subunits. In still further embodiments, the present invention provides ILRAP present in IL-2 receptor expressing cells, including but not limited to Kit 225 cells. The present invention also provides compositions comprising at least one ILRAP purified by immunoaffinity methods.

As indicated above, the present invention also provides methods for detecting the presence of ILRAP in a biological sample comprising the steps of contacting the sample with an antibody produced by the hybridoma having ATCC deposit No. PTA-82, under conditions such that the formation of a complex between the antibody and ILRAP is produced. In preferred embodiments, the presence of the complex indicates the presence of ILRAP.

The present invention further provides kits for detecting the presence of ILRAP in a biological sample. In preferred embodiments, the kit of the present invention comprises an antibody capable of recognizing ILRAP (*e.g.*, an antibody produced by the hybridoma having ATCC deposit No. PTA-82).

The present invention further provides methods for treating an animal having autoimmune disease. In preferred embodiments, the animal is a mammal having autoimmune disease. These methods comprising the administration of a therapeutically effective amount of an antibody capable of recognizing ILRAP (*e.g.*, an antibody produced by the hybridoma having ATCC deposit No. PTA-82) to the animal (*e.g.*, mammal).

The present invention also provides methods for treating an animal receiving an organ transplant. In preferred embodiments, the animal is a mammal receiving an organ transplant. These methods comprise administering a therapeutically effective amount of an antibody capable of recognizing ILRAP (*e.g.*, an antibody produced by a hybridoma having ATCC deposit No. PTA-82) to the animal (*e.g.*, a mammal).

The present invention still further provides methods for treating an animal receiving immunotherapy. In preferred embodiments, the animal is a mammal receiving immunotherapy. These methods comprise administering a therapeutically effective amount of an antibody capable of recognizing ILRAP (*e.g.*, an antibody produced by a hybridoma

having ATCC deposit No. PTA-82) to the animal (*e.g.*, the mammal. The present invention further provides methods for treating an animal having at least one tumor. In preferred embodiments, the animal is a mammal that has at least one tumor. In particular preferred embodiments, the animal (*e.g.*, mammal) with at least one tumor is also receiving immunotherapy.

DESCRIPTION OF THE FIGURES

Figure 1 shows the results of flow cytometric analyses of three cell lines using antibodies that identify each of the respective IL-2R subunits and the monoclonal antibody 5F7. The relative expression of each subunit as indicated by the relative mean fluorescence intensity of staining is shown on the x axis.

Figure 2 shows an SDS-PAGE analysis of an immunoprecipitation experiment to biochemically characterize ILRAP. Two major protein bands were immunoprecipitated from surface biotin labelled Kit 225 cells incubated with monoclonal antibody 5F7 conjugated to agarose beads (Lane 2). The predominant band exhibited an approximate size of 32 to 34 kDa, with a less intense band appearing at 26 to 28 kDa under reducing conditions. An additional faint band of approximate size of 55 kDa was co-precipitated. The faint band is similar in molecular size to the 55 kDa Tac peptide immunoprecipitated by the anti-IL-2Rα antibody anti-Tac (Lane 3). Non-specific immunoprecipitation was demonstrated using the murine IgMK myeloma antibody MOPC 104E conjugated to agarose beads (Lane 1).

Figure 3 shows co-modulation of ILRAPs with IL-2R upon incubation of IL-2R expressing cells with IL-2 at 37°C. Panel A shows flow cytometric analysis demonstrating that upon incubation with IL-2 at 37°C, HuT 102 cells lose their expression of ILRAP, whereas expression of an activation antigen receptor identified by the antibody 4B8 which is not IL-2R associated is not effected. Panel B shows that Kit 225 cells incubated in media alone at either 4°C or 37°C expressed both ILRAP and CD95, and incubation of these cells at 4°C with IL-2 did not effect the expression of these receptors. However, when cells were incubated with IL-2 at 37°C, allowing internalization of the IL-2 to proceed, ILRAP expression disappeared. The expression of CD95 which was not IL-2R associated was not eliminated.

Figure 4 shows a SDS-PAGE analysis demonstrating the co-precipitation of IL-2Rα and ILRAPs. When a film containing anti-ILRAP immunoprecipitated complex from Kit 225 cells was allowed to develop for an extended period of time, an additional diffuse protein band was revealed at ~55 kDa (Lane 2). This band migrated in a similar fashion to the major 55 kDa band immunoprecipitated by the anti-IL-2Rα antibody named anti-Tac (Lane 4). When the cell lysate was first precleared with anti-Tac conjugated beads before it was immunoprecipitated with anti-ILRAP conjugated beads, the 32-34 kDa and 26-28 kDa bands were retained, but the 55 kDa band was eliminated from the immunoprecipitated complex (Lane 3). The narrow discrete band remaining in Lane 3 after anti-IL-2Rα pre-clearing also appeared in the control (Lane 1) when non-immune mouse IgM conjugated beads were used for immunoprecipitation. This band was eliminated when the gel was run under non-reducing conditions, indicating that it is non-specific.

Figure 5 shows non-random association of ILRAP with IL-2Rα subunit on the surface of T cell line Kit 225 as determined by flow cytometric resonance energy transfer (FRET) analysis.

DEFINITIONS

To facilitate understanding of the invention, a number of terms are defined below.

"Associated peptide" as used herein refers to peptides that are bound directly or indirectly to other peptides. Associated peptides that are bound indirectly may have one or more other peptides bound between the two associated peptides. Peptides may be bound via peptide bonds, covalent bonds and non-covalent bonds.

"IL-2 receptor associated polypeptide" as used herein refers to polypeptides reactive with the monoclonal antibody produced by the hybridoma having ATCC deposit No. PTA-82 and that associate either directly or indirectly with the IL-2R.

A "variant" of ILRAP, as used herein, refers to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, (*e.g.*, replacement of leucine with isoleucine). More rarely, a variant may have "nonconservative" changes (*e.g.*, replacement of a glycine with a tryptophan). Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without

abolishing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software.

The term "biologically active," as used herein, refers to a protein or other biologically active molecules (*e.g.*, catalytic RNA) having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic ILRAP, or any oligopeptide or polynucleotide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The term "sample" as used herein is used in its broadest sense. A sample suspected of containing a human chromosome or sequences associated with a human chromosome may comprise a cell, chromosomes isolated from a cell (*e.g.*, a spread of metaphase chromosomes), genomic DNA (in solution or bound to a solid support such as for Southern blot analysis), RNA (in solution or bound to a solid support such as for Northern blot analysis), cDNA (in solution or bound to a solid support) and the like. A sample suspected of containing a protein may comprise a cell, a portion of a tissue, an extract containing one or more proteins and the like. A "biological sample" is a sample that has been obtained from an organism and encompasses any type of material (*e.g.*, including but not limited to body fluids, tissue, bone, bone marrow, etc.).

"In operable combination," "in operable order," and "operably linked" as used herein refer to the linkage of nucleic acid sequences in such a manner that a nucleic acid molecule capable of directing the transcription of a given gene and/or the synthesis of a desired protein molecule is produced. The term also refers to the linkage of amino acid sequences in such a manner so that a functional protein is produced.

As used herein, the terms "vector" and "vehicle" are used interchangeably in reference to nucleic acid molecules that transfer DNA segment(s) from one cell to another.

"Expression construct," "expression vector," and "plasmid" as used herein, refer to one or more recombinant DNA or RNA sequences containing a desired coding sequence operably linked to sequences necessary for the expression of the coding sequence in a cell or host organism (*e.g.*, a mammal). The sequence may be single or double stranded.

"Reporter construct," "reporter gene," and "reporter protein" as used herein, refer to DNA or amino acid sequences, as appropriate, that, when expressed in a host cell or organism, may be detected, measured or quantitated.

As used herein, the terms "purified" or "to purify" refers to the removal of one or

more (undesired) components from a sample. For example, where recombinant polypeptides are expressed in bacterial host cells, the polypeptides are purified by the removal of host cell proteins thereby increasing the percent of recombinant polypeptides in the sample.

As used herein, the term "partially purified" refers to the removal of a moderate portion of the contaminants of a sample to the extent that the substance of interest is recognizable by techniques known to those skilled in the art as accounting for a measurable amount of the mixture.

As used herein, the term "substantially purified" refers to molecules, (*e.g.*, nucleic or amino acid sequences) that are removed from their natural environment, isolated or separated, and are at least 60% free, preferably 75% free and more preferably 90% free from other components with which they are naturally associated. Furthermore, an "isolated polynucleotide" is a substantially purified polynucleotide.

As used herein "agent," "compound," and "drug" indicate a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian) cells or tissues that are suspected of having therapeutic properties. The compound, agent or drug may be purified, substantially purified or partially purified. Additionally, an "agent," "compound" or "drug" may be substantially pure (*i.e.,* comprised of essentially one component).

A compound is said to be "in a form suitable for administration to an animal" when the compound may be administered to an animal by any desired route (*e.g.*, oral, intravenous, subcutaneous, intramuscular, etc.). In particularly preferred embodiments, the compound or its active metabolites appear(s) in the blood of the animal. Administration of a compound to a pregnant female may result in delivery of the compound to the fetuses of the pregnant animal.

A "therapeutically effective" amount or dose refers to that amount of active ingredient, for example, anti-ILRAP antibodies or fragments thereof, agonists, antagonists or inhibitors of ILRAP, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals (*e.g.,* ED50 [the dose therapeutically effective in 50% of the population] and LD50 [the dose lethal to 50% of the population]. The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50.

Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

As used herein "agonist" refers to molecules or compounds which mimic the action of a "native" or "natural" compound. The present invention encompasses agonists that are homologous to these natural compounds in respect to conformation, charge or other characteristics, as well as compounds that are not homologous. Thus, agonists may or may not be recognized by, for example, receptors expressed on cell surfaces. In any event, regardless of whether the agonist is recognized by a natural compound in a manner similar to a "natural" compound or molecule, in some cases the agonist causes physiologic and/or biochemical changes within the cell (*i.e.*, such that the cell reacts to the presence of the agonist) in the same manner as if the natural compound was present.

As used herein "antagonist" refers to molecules or compounds which inhibit the action of a "native" or "natural" compound. As used herein, "antagonist" also encompasses compounds that are homologous to these natural compounds in respect to conformation, charge or other characteristics, as well as, compounds that are not homologous. Thus, antagonists are recognized by the same or different receptors or molecules as recognized by an agonist. Antagonists may have allosteric effects which prevent the action of an agonist (*e.g.*, by modifying a DNA adduct). In addition, in some cases, antagonists prevent the function of the agonist (*e.g.*, by blocking a DNA repair molecule).

As used herein, "patient" and "subject" refer to a human or other animal, such as a guinea pig or mouse and the like. In some preferred embodiments, a patient is treated using the methods and compositions of the present invention.

As used herein, "host" refers to a recipient cell or organism.

As used herein, "autoimmune disease" refers to any pathological condition in which an animal produces antibodies that recognize "self" antigens.

As used herein, "immunotherapy" refers to the use of therapy to improve the immune function of a subject. In particularly preferred embodiments, immunotherapy comprises the administration of compounds that stimulate the immune response to produce active immunity. However, it is not intended that the present invention be limited to any particular type of immunotherapy, as any therapy suitable for use with a particular subject is contemplated.

As used herein, the term "transplantation" refers the transfer of an organ (*i.e.*, organ transplant) and/or tissue from one human or non-human animal (*i.e.*, a "donor") to another human or non-human animal (*i.e.*, a recipient). It is not intended that the donor and/or recipient be limited to humans. Indeed, it is intended that the donor and/or recipient be of any species. In addition, the term encompasses the introduction of synthetic or materials prepared *in vitro*, for use in transplantation. For example, the term encompasses the use of synthetic or artificial substances (*e.g.*, bone and skin). In addition, the term encompasses the introduction of artificial devices or prosthetics (*e.g.*, heart valves, stents, joints, monitors, pacemakers, etc.). The term "transplant" refers to the material (organ, tissue, cells, artificial substances, devices, etc.) to be transplanted.

As used herein, the term "graft" refers to a portion of tissue or a collection of cells that is suitable for implantation or transplantation. It is intended that the term encompass any graft material and types, including but not limited to autologous, avascular, accordion, autodermic, autoepidermic, bone, fascicular, full-thickness, heterologous, heteroplastic, xenografts, nerve, muscle, tendon, ligament, synthetic, and other suitable grafts, including grafts obtained from biological material grown *in vitro*. The term "grafting" refers to the process of implanting or transplanting a graft.

As used herein, "apoptosis" refers to the generally recognized term for the morphological changes that are observed in a cell as the cell undergoes a non-accidental death.

"Programmed cell death" as used herein, refers to the genetically controlled process that is executed in a cell that has been induced to undergo apoptosis.

"Activation Induced Cell Death" and "AICD," as used herein, refers to the programmed cell death of T cells after activation of the T cells by an antigen.

"Antibody" as used herein, refers to defined as a glycoprotein produced by B cells and plasma cells that binds with high specificity to an antigen (usually, but not always, a peptide) or a structurally similar antigen, that generated its production. Antibodies may be produced by any of the known methodologies and may be either polyclonal or monoclonal.

The term "antigenic determinant" as used herein refers to that portion of an antigen that makes contact with a particular antibody (*i.e.*, an epitope). When a protein or fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to a given region or three-dimensional structure on the protein; these regions or structures are referred to as antigenic determinants. An antigenic determinant may compete with the intact antigen (*i.e.*, the "immunogen" used to elicit the immune response) for binding to an antibody.

The terms "specific binding" or specifically binding" when used in reference to the interaction of an antibody and a protein or peptide means that the interaction is dependent upon the presence of a particular structure (*i.e.*, the antigenic determinant or epitope) on the protein; in other words the antibody is recognizing and binding to a specific protein structure rather than to proteins in general. For example, if an antibody is specific for epitope "A," the presence of a protein containing epitope A (or free, unlabelled A) in a reaction containing labelled "A" and the antibody will reduce the amount of labelled A bound to the antibody.

The term "Western blot" refers to the analysis of protein(s) (or polypeptides) immobilized onto a support such as nitrocellulose or a membrane. The proteins are run on acrylamide gels to separate the proteins, followed by transfer of the protein from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized proteins are then exposed to antibodies with reactivity against an antigen of interest. The binding of the antibodies may be detected by various methods, including the use of radiolabelled antibodies.

The term "Southern blot," refers to the analysis of DNA on agarose or acrylamide gels to fractionate the DNA according to size followed by transfer of the DNA from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized DNA

is then probed with a labeled probe to detect DNA species complementary to the probe used. The DNA may be cleaved with restriction enzymes prior to electrophoresis. Following electrophoresis, the DNA may be partially depurinated and denatured prior to or during transfer to the solid support. Southern blots are a standard tool of molecular biologists (J. Sambrook *et al., Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, NY, pp 9.31-9.58 [1989]).

The term "Northern blot," as used herein refers to the analysis of RNA by electrophoresis of RNA on agarose gels to fractionate the RNA according to size followed by transfer of the RNA from the gel to a solid support, such as nitrocellulose or a nylon membrane. The immobilized RNA is then probed with a labeled probe to detect RNA species complementary to the probe used. Northern blots are a standard tool of molecular biologists (J. Sambrook, J. *et al.*, supra, pp 7.39-7.52 [1989]).

"Staining," as used herein, refers to any number of processes known to those in the field that are used to better visualize a specific component(s) and/or feature(s) of a cell or cells.

The terms "cancerous" and "cancer cell" refers to a cell undergoing early, intermediate or advanced stages of multi-step neoplastic progression as known in the art (*See e.g.*, Pitot, *in Fundamentals of Oncology*, Marcel Dekker (Ed.), New York pp 15-28 [1978]). The microscopic features of early, intermediate and advanced stages of neoplastic progression have been described. Cancer cells at each of the three stages of neoplastic progression generally have abnormal karyotypes, including translocations, inversion, deletions, isochromosomes, monosomies, and extra chromosomes. A cell in the early stages of malignant progression is referred to as a "hyperplastic cell" and is characterized by dividing without control and/or at a greater rate than a normal cell of the same cell type in the same tissue. Proliferation may be slow or rapid but continues unabated. A cell in the intermediate stages of neoplastic progression is referred to as a "dysplastic cell." A dysplastic cell resembles an immature epithelial cell, is generally spatially disorganized within the tissue and has lost its specialized structures and functions. For example, during the intermediate stages of neoplastic progression, an increasing percentage of the epithelium becomes composed of dysplastic cells. "Hyperplastic" and "dysplastic" cells are referred to as "pre-neoplastic" cells. In the advanced stages of neoplastic progression a dysplastic cell become a "neoplastic" cell. Neoplastic cells are typically invasive. Thus, they either invade adjacent tissues, or are shed from the primary site and circulate through

the blood and lymph to other locations in the body where they initiate secondary cancers. The term "cancer" or "neoplasia" refers to a plurality of cancer cells.

"Nucleic acid sequence," "nucleotide sequence" and "polynucleotide sequence" as used herein refer to an oligonucleotide or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be single- or double-stranded, and represent the sense or antisense strand.

As used herein, the terms "oligonucleotides" and "oligomers" refer to a nucleic acid sequence of at least about 10 nucleotides and as many as about 60 nucleotides, preferably about 15 to 30 nucleotides, and more preferably about 20-25 nucleotides, which can be used as a probe or amplimer.

The term "nucleotide sequence of interest" refers to any nucleotide sequence, the manipulation of which may be deemed desirable for any reason, by one of ordinary skill in the art. Such nucleotide sequences include, but are not limited to, coding sequences of structural genes (*e.g.*, reporter genes, selection marker genes, oncogenes, drug resistance genes, growth factors, *etc.*), and of non-coding regulatory sequences that do not encode an mRNA or protein product (*e.g.*, promoter sequence, enhancer sequence, polyadenylation sequence, termination sequence, *etc.*).

The term "wild-type" refers to a gene or gene product which has the characteristics of that gene or gene product when isolated from a naturally occurring source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designed the "normal" or "wild-type" form of the gene. In contrast, the term "modified" or "mutant" refers to a gene or gene product which displays modifications in sequence and or functional properties (*i.e.*, altered characteristics) when compared to the wild-type gene or gene product. It is noted that naturally-occurring mutants can be isolated; these are identified by the fact that they have altered characteristics when compared to the wild-type gene or gene product.

"Amino acid sequence," "polypeptide sequence," "peptide sequence," and "peptide" are used interchangeably herein to refer to a sequence of amino acids.

The term "portion" when used in reference to a nucleotide sequence refers to fragments of that nucleotide sequence. The fragments may range in size from 5 nucleotide residues to the entire nucleotide sequence minus one nucleic acid residue. The term "portion" when used in reference to an amino acid sequence refers to fragments of the amino acid sequence. The fragments may range in size from 3 amino acids to the entire

amino acid sequence minus one amino acid residue.

An oligonucleotide sequence which is a "homolog" of a first nucleotide sequence is defined herein as an oligonucleotide sequence which exhibits greater than or equal to 50% identity, and more preferably greater than or equal to 70% identity, to the first nucleotide sequence when sequences having a length of 10 bp or larger are compared.

DNA molecules are said to have "5' ends" and "3' ends" because mononucleotides are reacted to make oligonucleotides in a manner such that the 5' phosphate of one mononucleotide pentose ring is attached to the 3' oxygen of its neighbor in one direction via a phosphodiester linkage. Therefore, an end of an oligonucleotide is referred to as the "5' end" if its 5' phosphate is not linked to the 3' oxygen of a mononucleotide pentose ring. An end of an oligonucleotide is referred to as the "3' end" if its 3' oxygen is not linked to a 5' phosphate of another mononucleotide pentose ring. As used herein, a nucleic acid sequence, even if internal to a larger oligonucleotide, also may be said to have 5' and 3' ends. In either a linear or circular DNA molecule, discrete elements are referred to as being "upstream" or 5' of the "downstream" or 3' elements. This terminology reflects that transcription proceeds in a 5' to 3' direction along the DNA strand. The promoter and enhancer elements which direct transcription of a linked gene are generally located 5' or upstream of the coding region. However, enhancer elements can exert their effect even when located 3' of the promoter element and the coding region. Transcription termination and polyadenylation signals are located 3' or downstream of the coding region.

The term "cloning" as used herein, refers to the process of isolating a nucleotide sequence from a nucleotide library, cell or organism for replication by recombinant techniques.

The term "recombinant DNA molecule" as used herein refers to a DNA molecule which is comprised of segments of DNA joined together by means of molecular biological techniques.

The term "recombinant protein" or "recombinant polypeptide" as used herein refers to a protein molecule which is expressed using a recombinant DNA molecule.

The term "transfection" as used herein refers to the introduction of foreign DNA into cells. Transfection may be accomplished by a variety of means known to the art including calcium phosphate-DNA co-precipitation, DEAE-dextran-mediated transfection. polybrene-mediated transfection, electroporation, microinjection, liposome fusion,

lipofection, protoplast fusion, retroviral infection, biolistics (*i.e.*, particle bombardment) and the like.

As used herein, the terms "complementary" or "complementarity" are used in reference to "polynucleotides" and "oligonucleotides" (which are interchangeable terms that refer to a sequence of nucleotides) related by the base-pairing rules. For example, the sequence "5'-CAGT-3'," is complementary to the sequence "5'-ACTG-3'." Complementarity can be "partial" or "total." "Partial" complementarity is where one or more nucleic acid bases is not matched according to the base pairing rules. "Total" or "complete" complementarity between nucleic acids is where each and every nucleic acid base is matched with another base under the base pairing rules. The degree of complementarity between nucleic acid strands may have significant effects on the efficiency and strength of hybridization between nucleic acid strands. This may be of particular importance in amplification reactions, as well as detection methods which depend upon binding between nucleic acids.

The terms "homology" and "homologous" as used herein in reference to nucleotide sequences refer to a degree of complementarity with other nucleotide sequences. There may be partial homology or complete homology (*i.e.*, identity). A nucleotide sequence which is partially complementary (*i.e.*, "substantially homologous") to a nucleic acid sequence is one that at least partially inhibits a completely complementary sequence from hybridizing to a target nucleic acid sequence. The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or Northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe will compete for and inhibit the binding (*i.e.*, the hybridization) of a completely homologous sequence to a target sequence under conditions of low stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (*i.e.*, selective) interaction. The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (*e.g.*, less than about 30% identity); in the absence of non-specific binding the probe will not hybridize to the second non-complementary target.

As used herein the term "stringency" is used in reference to the conditions of temperature, ionic strength, and the presence of other compounds such as organic solvents, under which nucleic acid hybridizations are conducted. "Stringency" typically occurs in a range from about T_m°C to about 20°C to 25°C below T_m. As will be understood by those of skill in the art, a stringent hybridization can be used to identify or detect identical polynucleotide sequences or to identify or detect similar or related polynucleotide sequences. Under "stringent conditions" the nucleotide sequence portions thereof, will hybridize to its exact complement and closely related sequences.

Low stringency conditions comprise conditions equivalent to binding or hybridization at 68°C in a solution consisting of 5X SSPE (43.8 g/l NaCl, 6.9 g/l $NaH_2PO_4 \cdot H_2O$ and 1.85 g/l EDTA, pH adjusted to 7.4 with NaOH), 0.1 % SDS, 5X Denhardt's reagent (50X Denhardt's contains per 500 ml: 5 g Ficoll (Type 400, Pharmacia), 5 g BSA (Fraction V; Sigma)) and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 2.0X SSPE, 0.1% SDS at room temperature when a probe of about 100 to about 1000 nucleotides in length is employed.

It is well known in the art that numerous equivalent conditions may be employed to comprise low stringency conditions; factors such as the length and nature (DNA, RNA, base composition) of the probe and nature of the target (DNA, RNA, base composition, present in solution or immobilized, *etc.*) and the concentration of the salts and other components (*e.g.*, the presence or absence of formamide, dextran sulfate, polyethylene glycol), as well as components of the hybridization solution may be varied to generate conditions of low stringency hybridization different from, but equivalent to, the above listed conditions. In addition, conditions which promote hybridization under conditions of high stringency (*e.g.*, increasing the temperature of the hybridization and/or wash steps, the use of formamide in the hybridization solution, *etc.*) are well known in the art. High stringency conditions, when used in reference to nucleic acid hybridization, comprise conditions equivalent to binding or hybridization at 68°C in a solution consisting of 5X SSPE, 1 % SDS, 5X Denhardt's reagent and 100 µg/ml denatured salmon sperm DNA followed by washing in a solution comprising 0.1X SSPE and 0.1 % SDS at 68°C when a probe of about 100 to about 1000 nucleotides in length is employed.

When used in reference to a double-stranded nucleic acid sequence such as a cDNA or genomic clone, the term "substantially homologous" refers to any probe which can hybridize either partially or completely to either or both strands of the double-stranded

nucleic acid sequence under conditions of low stringency as described above.

When used in reference to a single-stranded nucleic acid sequence, the term "substantially homologous" refers to any probe which can hybridize (*i.e.*, it is the complement of) the single-stranded nucleic acid sequence under conditions of low stringency as described above.

As used herein, the term "hybridization" is used in reference to the pairing of complementary nucleic acids using any process by which a strand of nucleic acid joins with a complementary strand through base pairing to form a hybridization complex. Hybridization and the strength of hybridization (*i.e.*, the strength of the association between the nucleic acids) is impacted by such factors as the degree of complementarity between the nucleic acids, stringency of the conditions involved, the T_m of the formed hybrid, and the G:C ratio within the nucleic acids.

As used herein the term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bounds between complementary G and C bases and between complementary A and T bases; these hydrogen bonds may be further stabilized by base stacking interactions. The two complementary nucleic acid sequences hydrogen bond in an antiparallel configuration. A hybridization complex may be formed in solution (*e.g.*, C_0t or R_0t analysis) or between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized to a solid support (*e.g.*, a nylon membrane or a nitrocellulose filter as employed in Southern and Northern blotting, dot blotting or a glass slide as employed in *in situ* hybridization, including FISH (fluorescent *in situ* hybridization)).

As used herein, the term "T_m" is used in reference to the "melting temperature." The melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. The equation for calculating the T_m of nucleic acids is well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation: $T_m = 81.5 + 0.41(\%\ G + C)$, when a nucleic acid is in aqueous solution at 1 M NaCl (*see e.g.*, Anderson and Young, Quantitative Filter Hybridization, *in Nucleic Acid Hybridization* [1985]). Other references include more sophisticated computations which take structural as well as sequence characteristics into account for the calculation of T_m.

The term "heterologous nucleic acid sequence" or "heterologous DNA" are used interchangeably to refer to a nucleotide sequence which is ligated to a nucleic acid sequence to which it is not ligated in nature, or to which it is ligated at a different location in nature. Heterologous DNA is not endogenous to the cell into which it is introduced, but has been obtained from another cell. Generally, although not necessarily, such heterologous DNA encodes RNA and proteins that are not normally produced by the cell into which it is expressed. Examples of heterologous DNA include reporter genes, transcriptional and translational regulatory sequences, selectable marker proteins (*e.g.*, proteins which confer drug resistance), *etc.*

"Amplification" is defined herein as the production of additional copies of a nucleic acid sequence and is generally carried out using polymerase chain reaction technologies well known in the art (see, *e.g.*, Dieffenbach and Dveksler, *PCR Primer, a Laboratory Manual*, Cold Spring Harbor Press, Plainview NY [1995]). As used herein, the term "polymerase chain reaction" ("PCR") refers to the methods of U.S. Patent Nos. 4,683,195, 4,683,202, and 4,965,188, all of which are hereby incorporated by reference, which describe a method for increasing the concentration of a segment of a target sequence in a mixture of genomic DNA without cloning or purification. The length of the amplified segment of the desired target sequence is determined by the relative positions of two oligonucleotide primers with respect to each other, and therefore, this length is a controllable parameter. By virtue of the repeating aspect of the process, the method is referred to as the "polymerase chain reaction" (hereinafter "PCR"). Because the desired amplified segments of the target sequence become the predominant sequences (in terms of concentration) in the mixture, they are said to be "PCR amplified."

With PCR, it is possible to amplify a single copy of a specific target sequence in genomic DNA to a level detectable by several different methodologies (*e.g.*, hybridization with a labeled probe; incorporation of biotinylated primers followed by avidin-enzyme conjugate detection; incorporation of ^{32}P-labeled deoxynucleotide triphosphates, such as dCTP or dATP, into the amplified segment). In addition to genomic DNA, any oligonucleotide sequence can be amplified with the appropriate set of primer molecules. In particular, the amplified segments created by the PCR process itself are, themselves, efficient templates for subsequent PCR amplifications.

The terms "reverse transcription polymerase chain reaction" and "RT-PCR" refer to a method for reverse transcription of an RNA sequence to generate a mixture of cDNA sequences, followed by increasing the concentration of a desired segment of the transcribed cDNA sequences in the mixture without cloning or purification. Typically, RNA is reverse transcribed using a single primer (*e.g.*, an oligo-dT primer) prior to PCR amplification of the desired segment of the transcribed DNA using two primers.

As used herein, the term "primer" refers to an oligonucleotide, whether occurring naturally as in a purified restriction digest or produced synthetically, which is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product which is complementary to a nucleic acid strand is induced, (*i.e.*, in the presence of nucleotides and of an inducing agent such as DNA polymerase and at a suitable temperature and pH). The primer is preferably single stranded for maximum efficiency in amplification, but may alternatively be double stranded. If double stranded, the primer is first treated to separate its strands before being used to prepare extension products. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent. The exact lengths of the primers will depend on many factors, including temperature, source of primer and the use of the method.

As used herein, the term "probe" refers to an oligonucleotide (*i.e.*, a sequence of nucleotides), whether occurring naturally as in a purified restriction digest or produced synthetically, recombinantly or by PCR amplification, which is capable of hybridizing to another oligonucleotide of interest. A probe may be single-stranded or double-stranded. Probes are useful in the detection, identification and isolation of particular gene sequences. It is contemplated that any probe used in the present invention will be labeled with any "reporter molecule," so that it is detectable in any detection system, including, but not limited to enzyme (*e.g.*, ELISA, as well as enzyme-based histochemical assays), fluorescent, radioactive, and luminescent systems. It is not intended that the present invention be limited to any particular detection system or label.

As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to bacterial enzymes, each of which cut double- or single-stranded DNA at or near a specific nucleotide sequence.

As used herein, the term "an oligonucleotide having a nucleotide sequence encoding a gene" means a nucleic acid sequence comprising the coding region of a gene, *i.e.* the

nucleic acid sequence which encodes a gene product. The coding region may be present in either a cDNA, genomic DNA or RNA form. When present in a DNA form, the oligonucleotide may be single-stranded (*i.e.*, the sense strand) or double-stranded. Suitable control elements such as enhancers, promoters, splice junctions, polyadenylation signals, *etc.* may be placed in close proximity to the coding region of the gene if needed to permit proper initiation of transcription and/or correct processing of the primary RNA transcript. Alternatively, the coding region utilized in the expression vectors of the present invention may contain endogenous enhancers, splice junctions, intervening sequences, polyadenylation signals, *etc.* or a combination of both endogenous and exogenous control elements.

Transcriptional control signals in eukaryotes comprise "enhancer" elements. Enhancers consist of short arrays of DNA sequences that interact specifically with cellular proteins involved in transcription (Maniatis *et al.*, Science 236:1237 [1987]). Enhancer elements have been isolated from a variety of eukaryotic sources including genes in plant, yeast, insect and mammalian cells and viruses. The selection of a particular enhancer depends on what cell type is to be used to express the protein of interest.

The presence of "splicing signals" on an expression vector often results in higher levels of expression of the recombinant transcript. Splicing signals mediate the removal of introns from the primary RNA transcript and consist of a splice donor and acceptor site (Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, New York, pp. 16.7-16.8 [1989]). A commonly used splice donor and acceptor site is the splice junction from the 16S RNA of SV40.

Efficient expression of recombinant DNA sequences in eukaryotic cells requires expression of signals directing the efficient termination and polyadenylation of the resulting transcript. Transcription termination signals are generally found downstream of the polyadenylation signal and are a few hundred nucleotides in length. The term "poly A site" or "poly A sequence" as used herein denotes a DNA sequence which directs both the termination and polyadenylation of the nascent RNA transcript. Efficient polyadenylation of the recombinant transcript is desirable as transcripts lacking a poly A tail are unstable and are rapidly degraded. The poly A signal utilized in an expression vector may be "heterologous" or "endogenous." An endogenous poly A signal is one that is found naturally at the 3' end of the coding region of a given gene in the genome. A

heterologous poly A signal is one which is isolated from one gene and placed 3' of another gene.

The term "promoter," "promoter element," or "promoter sequence" as used herein, refers to a DNA sequence which when placed at the 5' end of (*i.e.*, precedes) an oligonucleotide sequence is capable of controlling the transcription of the oligonucleotide sequence into mRNA. A promoter is typically located 5' (*i.e.*, upstream) of an oligonucleotide sequence whose transcription into mRNA it controls, and provides a site for specific binding by RNA polymerase and for initiation of transcription.

The term "promoter activity" when made in reference to a nucleic acid sequence refers to the ability of the nucleic acid sequence to initiate transcription of an oligonucleotide sequence into mRNA.

As used herein, the terms "nucleic acid molecule encoding," "nucleotide encoding." "DNA sequence encoding," and "DNA encoding" refer to the order or sequence of deoxyribonucleotides along a strand of deoxyribonucleic acid. The order of these deoxyribonucleotides determines the order of amino acids along the polypeptide (protein) chain. The DNA sequence thus codes for the amino acid sequence.

The term "isolated" when used in relation to a nucleic acid, as in "an isolated oligonucleotide" refers to a nucleic acid sequence that is separated from at least one contaminant nucleic acid with which it is ordinarily associated in its natural source. Isolated nucleic acid is nucleic acid present in a form or setting that is different from that in which it is found in nature. In contrast, non-isolated nucleic acids are nucleic acids such as DNA and RNA which are found in the state they exist in nature. For example, a given DNA sequence (*e.g.*, a gene) is found on the host cell chromosome in proximity to neighboring genes; RNA sequences, such as a specific mRNA sequence encoding a specific protein, are found in the cell as a mixture with numerous other mRNAs which encode a multitude of proteins. However, isolated nucleic acid encoding a polypeptide of interest includes, by way of example, such nucleic acid in cells ordinarily expressing the polypeptide of interest where the nucleic acid is in a chromosomal or extrachromosomal location different from that of natural cells, or is otherwise flanked by a different nucleic acid sequence than that found in nature. The isolated nucleic acid or oligonucleotide may be present in single-stranded or double-stranded form. Isolated nucleic acid can be readily identified (if desired) by a variety of techniques (*e.g.*, hybridization, dot blotting, etc.). When an isolated nucleic acid or oligonucleotide is to be utilized to express a protein, the

oligonucleotide will contain at a minimum the sense or coding strand (*i.e.*, the oligonucleotide may be single-stranded). Alternatively, it may contain both the sense and anti-sense strands (*i.e.*, the oligonucleotide may be double-stranded).

As used herein the term "coding region" when used in reference to a structural gene refers to the nucleotide sequences which encode the amino acids found in the nascent polypeptide as a result of translation of a mRNA molecule. The coding region is bounded, in eukaryotes, on the 5' side by the nucleotide triplet "ATG" which encodes the initiator methionine and on the 3' side by one of the three triplets which specify stop codons (*i.e.*, TAA, TAG, TGA).

As used herein, the term "structural gene" or "structural nucleotide sequence" refers to a DNA sequence coding for RNA or a protein which does not control the expression of other genes. In contrast, a "regulatory gene" or "regulatory sequence" is a structural gene which encodes products (*e.g.*, transcription factors) which control the expression of other genes.

As used herein, the term "regulatory element" refers to a genetic element which controls some aspect of the expression of nucleic acid sequences. For example, a promoter is a regulatory element which facilitates the initiation of transcription of an operably linked coding region. Other regulatory elements include splicing signals, polyadenylation signals, termination signals, *etc.*

As used herein, the term "gene" means the deoxyribonucleotide sequences comprising the coding region of a structural gene. A "gene" may also include non-translated sequences located adjacent to the coding region on both the 5' and 3' ends such that the gene corresponds to the length of the full-length mRNA. The sequences which are located 5' of the coding region and which are present on the mRNA are referred to as 5' non-translated sequences. The sequences which are located 3' or downstream of the coding region and which are present on the mRNA are referred to as 3' non-translated sequences. The term "gene" encompasses both cDNA and genomic forms of a gene. A genomic form or clone of a gene contains the coding region interrupted with non-coding sequences termed "introns" or "intervening regions" or "intervening sequences." Introns are segments of a gene which are transcribed into heterogenous nuclear RNA (hnRNA); introns may contain regulatory elements such as enhancers. Introns are removed or "spliced out" from the nuclear or primary transcript; introns therefore are absent in the messenger RNA

(mRNA) transcript. The mRNA functions during translation to specify the sequence or order of amino acids in a nascent polypeptide.

In addition to containing introns, genomic forms of a gene may also include sequences located on both the 5' and 3' end of the sequences which are present on the RNA transcript. These sequences are referred to as "flanking" sequences or regions (these flanking sequences are located 5' or 3' to the non-translated sequences present on the mRNA transcript). The 5' flanking region may contain regulatory sequences such as promoters and enhancers which control or influence the transcription of the gene. The 3' flanking region may contain sequences which direct the termination of transcription, post-transcriptional cleavage and polyadenylation.

A "non-human animal" refers to any animal which is not a human and includes vertebrates such as rodents, non-human primates, ovines, bovines, ruminants, lagomorphs, porcines, caprines, equines, canines, felines, aves, etc. Preferred non-human animals are selected from the order Rodentia.

As used herein, the term "cell culture" refers to any *in vitro* culture of cells. Included within this term are continuous cell lines (*e.g.*, with an immortal phenotype), primary cell cultures, finite cell lines (*e.g.*, non-transformed cells), and any other cell population maintained *in vitro*.

A "transformed cell" is a cell or cell line that has acquired the ability to grow in cell culture for many multiple generations, the ability to grow in soft agar and the ability to not have cell growth inhibited by cell-to-cell contact. In this regard, transformation refers to the introduction of foreign genetic material into a cell or organism. Transformation may be accomplished by any method known which permits the successful introduction of nucleic acids into cells and which results in the expression of the introduced nucleic acid. "Transformation" includes but is not limited to such methods as transfection, microinjection, electroporation, and lipofection (liposome-mediated gene transfer). Transformation may be accomplished through use of any expression vector. For example, the use of baculovirus to introduce foreign nucleic acid into insect cells is contemplated. The term "transformation" also includes methods such as P-element mediated germline transformation of whole insects. Additionally, transformation refers to cells that have been transformed naturally, usually through genetic mutation.

As used herein, the term "kit" is used in reference to a combination of reagents and other materials. It is contemplated that the kit may include reagents such as anti-ILRAP antibodies, control ILRAP protein, as well as testing containers (*e.g.*, microtiter plates, etc.). It is not intended that the term "kit" be limited to a particular combination of reagents and/or other materials.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to two polypeptides which are associated with the interleukin-2 receptor (IL-2R) in the plasma membrane of cells expressing the IL-2R. These two polypeptides, designated interleukin-2 receptor associated polypeptides, have molecular weights of about 32,000 to 34,000 daltons and 26,000 to 28,000 daltons, respectively, and are reactive with the monoclonal antibody 5F7. The invention also provides antibodies specific for these polypeptides and methods for use of these antibodies in various methods, including but not limited to the detection of these polypeptides and for the isolation and purification of these polypeptides from biological samples.

In particular, based on a strategy that whereas an antibody directed toward IL-2 might have a complementary configuration to IL-2 and would bind to this cytokine, and an antibody to the idiotype of such an anti-IL-2 antibody might have a shape similar to that of IL-2 itself and thus might bind to an IL-2R associated protein, the ILRAPs were identified using an antibody directed toward the idiotype of the anti-IL-2 antibody 7B1. This anti-idiotypic monoclonal antibody, designated 5F7, immunoprecipitated two polypeptides having molecular weights of about 32,000 - 34,000 daltons and 26,000 - 28,000 daltons, respectively, from cells bearing the high affinity IL-2R such as the mature T cell lines HuT 102 and Kit 225, from cells expressing the IL2-Rβ and γ subunits such as the MT1 cells, and from cells which express the IL2-Rα and γ subunits and which can be induced to express the α subunit such as the NK cell line YT.

The molecular weights of the ILRAPs were determined by SDS-PAGE. The complex immunoprecipitated with the 5F7 antibody was electrophoresed through a 12.5% SDS-PAGE gel with standard molecular weight markers. The molecular weights of the ILRAPs were estimated based on the molecular weights detected in the marker lane. The ILRAPs coprecipitated with the IL-2R α subunit identified by the anti-Tac antibody, as shown in the immunoprecipitation analyses of Example 4. In addition, the ILRAPs were found to be non-randomly associated with the IL-2R α subunit on the surface of IL-2R

expressing T cell lines, as shown by flow cytometric resonance energy transfer (FRET) measurements (*See,* Example 4). Furthermore, the ILRAPs co-internalize with the IL-2R β chain of the high affinity receptor in IL-2R expressing cells upon IL-2 addition at 37°C (*See,* Example 3).

The invention also provides the monoclonal antibody 5F7, and the murine hybridoma cell line CKG Hd5F7 which produces 5F7. The ATCC has assigned the deposit number PTA-82 to this hybridoma. The monoclonal antibody 5F7 is specific to the ILRAPs and does not react immunologically with the α, β and γ subunits and of the IL-2R in a direct fashion; the appearance of the 55 kDa band with the ILRAPs is a co-precipitation of the Tac peptide from the complex.

The present invention further provides anti-idiotypic antibodies to the anti-IL-2 monoclonal antibody 7B1. Anti-idiotypic antibodies to 7B1 may be produced by immunizing animals with the antibody 7B1 (*i.e.,* as described in Example 1). These anti-idiotypic antibodies to 7B1 may be used to screen for additional IL-2R associated proteins.

The present invention also provides methods to the use of the antibodies of the invention for the detection of ILRAPs in biological samples. In one embodiment, a biological sample is contacted with a diagnostically effective amount (*i.e.,* a concentration that is suitable and sufficient for use in diagnostic tests) of the antibody of this invention under conditions which will allow the formation of an immunological complex between the antibody and the ILRAP antigen that may be present in the sample. The formation of an immunological complex which indicates the presence of ILRAP in the sample, is then detected by an immunoassay. Such assays include, but are not limited to, radioimmunoassays, Western blot assay, immunofluorescent assay, enzyme immunoassay, chemiluminescent assay, immunohistochemical assay, and the like.

The present invention further provides diagnostic kits. In particularly preferred embodiments, the kits comprise the antibody of the invention. Such diagnostic kits may include other reagents and material required (*e.g.,* for immunoassays).

The present invention also provides methods for the antibodies of the invention for the isolation and purification of the polypeptides of the invention. The ILRAPs can be isolated and purified using techniques known in the art. Cell lines which can be used to isolate the ILRAPs include, but are not limited to, HuT 102 and Kit 225. In one embodiment, Kit 225 cells grown in 10% fetal bovine serum/RPMI media are collected by

centrifugation, washed in phosphate buffered saline, and solubilized in a lysis buffer containing 1% Triton X-100. The solubilized lysates are then passed through an anti-ILRAP (mab5F7) immunoaffinity column. The immunoaffinity columns can be generated using methods known in the art. After non-specific proteins are removed from the column by extensive washing, ILRAPs are eluted by decreasing the pH of the column below pH 4. The eluted ILRAPs are then concentrated and further purified by SDS-PAGE. In addition, since the IL-2R associated polypeptides of the invention may mediate ACID, it is contemplated that the antibodies of the invention will find use in various therapies. For example, if the process of self tolerance fails due to failure of steps beyond that of the IL-2R, autoimmune disease may develop, it is contemplated that the antibodies of the present invention will find use in eliminating T cells expressing the IL-2R associated polypeptides of the invention in animals (*e.g.*, mammals) with autoimmune disease. Furthermore, as these self reactive T cells may also be involved in patients receiving organ allografts or those suffering graft versus-host-disease following a bone marrow transplant, the present invention also provides antibodies suitable for use in the elimination of T cells expressing the ILRAPs in animals (*e.g.*, mammals) receiving organ transplants.

Alternatively, the antibody of the invention may be used to treat tumor-bearing animals (*e.g.*, mammals) receiving immunotherapy. In most cases, tumor-bearing mammals receiving immunotherapy develop an immune response to cancer cells which are aborted in the one or two weeks following the development of cancer or the introduction of the cancer cells before all of them are eliminated. In these situations, signaling mediated by the IL-2R through IL-2R associated proteins must be eliminated to prevent the tumor-reactive T cells from dying so that they can eliminate the cancer. Therefore, the present invention also provides antibodies suitable for maintaining the immune response of tumor reactive T cells involved in the host response to antigens on cancer cells in tumor-bearing animals (*e.g.*, mammals). In particular, it is contemplated that approaches to maintain ILRAP expressing cells are of value in association with the use of anti-cancer vaccines.

When used in therapy, the antibodies of the invention are suitable for administration via any one of several routes including, but not limited to intravenous, intraperitoneal, intramuscular, and subcutaneous injections. A preferred route of administration is intravenous. Those of skill in the art are readily able to determine the dosage to be

administered for any particular treatment protocol, dependent upon patient factors (*e.g.*, the sex, age and clinical status of the patient), as well as other factors related to the form of the dose (*i.e.*, suspensions for oral or injectable use, gels or creams, solids, etc.). Thus, it is not intended that the present invention be limited to any particular route of administration nor type of composition comprising the antibodies of the present invention.

EXPERIMENTAL

The following examples serve to illustrate certain preferred embodiments and aspects of the present invention and are not to be constructed as limiting the scope thereof.

In the experimental disclosure which follows, the following abbreviations apply: °C (degrees Centigrade); H_2O (water); aa (amino acid); bp (base pair); kb (kilobase pair); kDa (kilodaltons); gm (grams); µg (micrograms); mg (milligrams); ng (nanograms); µl (microliters); ml (milliliters); mm (millimeters); nm (nanometers); µm (micrometer); M (molar); mM (millimolar); µM (micromolar); nM (nanomolar); sec (seconds); min(s) (minute/minutes); hr(s) and h (hour/hours); ab (antibody); mAB (monoclonal antibody); PBS (phosphate buffered saline); PAGE (polyacrylamide gel electrophoresis); SDS (sodium dodecyl sulfate); SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis); FBS (fetal bovine serum); Tris (tris(hydroxymethyl)aminomethane); EDTA (ethylenediaminetetraacetic acid); w/v (weight to volume); v/v (volume to volume); RT (room temperature); FACS (fluorescence activated cell sorter); Millipore (Millipore, Bedford, MA); Sigma (Sigma Chemical Co., St. Louis, MO); Pharmacia (Pharmacia Biotech, Uppsala, Sweden); Becton Dickinson (San Jose, CA); Life Technologies (Bethesda, MD); ATCC (Manassas, VA); Pierce (Rockford, Illinois); Calbiochem (La Jolla, CA); Spectrum Laboratories (Houston, TX); Hoffmann-LaRoche (Nutley, NJ); Novex (San Diego, CA); Tropix, Inc. (Bedford, MA); PharMingen (San Diego, CA); Caltag (Burlingame, CA).

EXAMPLE 1

Antibody Production

Although an understanding of the mechanism is not necessary to use the present invention, this Example describes experiments to produce and characterize various antibodies used in these Examples. The alpha chain of the human interleukin 2 receptor (IL-2R) was detected by anti-Tac, a murine monoclonal antibody (Uchiyama T, *et al.*, "A

monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. I. Production of anti-Tac monoclonal antibody and distribution of Tac (+) cells" *J Immunol* 129:2066-2068, 1981; Depper JM, *et al.*, "Blockade of the interleukin-2 receptor by anti-Tac antibody: inhibition of human lymphocyte activation" *Immunol* 131: 690-696, 1983; Leonard WJ, *et al.* "Characterization of the human receptor for T-cell growth factor" *Proc Natl Acad Sci USA*, 80:6957-6961, 1983; Leonard WJ, *et al.*, "A monoclonal antibody that appears to recognize the receptor for human T-cell growth factor, partial characterization of the receptor" *Nature* 300:267-269, 1982; Leonard WJ. *et al.*, "Molecular cloning and expression of cDNAs for the human interleukin-2 receptor" *Nature* 311:626-631, 1984). The β chain and gamma chain were detected by antibodies Mikβ1 and TUGH4 (PharMingen), respectively. The antibody 4B8 recognizes an activation antigen on human cells and was developed during the development of the present invention. Mouse IgG1 and IgG2a nonimmune isotype controls were from Becton Dickinson Biosciences, anti-CD95 (PharMingen), and fluorescein conjugated F(ab)2 goat anti-mouse IgG+IgM were from Caltag. 7B1, a murine monoclonal antibody directed against human interleukin-2 (IL-2), was used as the immunogen for the production of the hybridoma CKG Hd5F7 (ATCC deposit No. PTA-82). 7B1 is a murine monoclonal, IgG2a antibody that binds to a fragment of human IL-2 comprising amino acids 9 to 19 of the IL-2 molecule and neutralizes IL-2 bioactivity.

The monoclonal antibody CKG Hd5F7 was produced as described below. Anti-idiotypic antibodies directed against murine monoclonal antibodies which recognize human IL-2 were raised in the mouse according to the method of Bluestone (Bluestone, J.A. *et al.*, *J Immunol* 1982, 126:1393-7). In brief, murine monoclonal 7B1 was purified from ascites by IL-2 affinity chromatography, concentrated to 1 mg/ml, and mixed with an equal volume of 1 mg/ml keyhole limpet hemocyanin (KLH) (Calbiochem) in phosphate buffered saline (PBS). Gluteraldehyde at a final concentration of 0.1% was added to the solution to couple the KLH to the antibody. The reaction was allowed to proceed at room temperature with stirring for 30 minutes until the solution became opalescent and was then stopped with 0.2 M final concentration lysine and dialyzed overnight against PBS.

Balb/c mice were injected intradermally with 100 μg of coupled antibody in complete Freund's adjuvant at multiple sites and boosted at day 5 with 100 μg of coupled antibody in incomplete Freund's adjuvant and at days 19, 26, and 33 with coupled antibody in PBS. Three days following the final boost, spleens were harvested and fused with the

murine myeloma cell line P3X63Ag8.653, a non-antibody secreting subclone of the parent myeloma line P3X-63-AG8, according to the method of Nowinski (Nowinski, R.C., *et al.*, "The isolation of hybrid cell lines producing monoclonal antibodies against the p15(E) protein of ecotropic murine leukemia viruses" *Virol* 93:111-126, 1979). Hybridomas were selected for further study based on the ability of their secreted antibody to bind to the cell lines Hut 102 (high affinity IL-2R bearing cells) and YT-1 (intermediate affinity IL-2R bearing cells) but not to Molt4 or CEM as detected using flow cytometry as discussed below. The hybridoma Hd5F7 was subcloned 4 times by limiting dilution. The isotype of the antibody produced by this line was determined to be IgM kappa.

Purified murine monoclonal antibody 5F7 (from hybridoma CKG Hd5F7) was prepared for FACS distribution studies, cointernalization studies, and coupling to beads for immunoprecipitation studies from hybridoma culture supernatant. The IgM monoclonal was isolated using ammonium sulphate precipitation followed by mannan binding protein affinity column chromatography (Pierce) according to manufacturer's instructions. MOPC 104E, a non-immune murine IgM myeloma used as a control, was similarly purified. The 5F7 antibody secreted into conditioned media by hybridoma cells cultured in a serum-free hollow fiber bioreactor system was harvested, purified by mannan binding protein affinity column chromatography, concentrated using a Biomax centrifugal filter device (Millipore Corp.) and coupled to agarose beads without further purification for use as affinity columns for solubilized cellular ILRAP isolation.

EXAMPLE 2

Cell Lines and Cell Culture

Although an understanding of the mechanism is not necessary to use the present invention, this Example describes experiments to characterize and maintain the various cell lines used in these Examples. HuT 102 is an HTLV1 (human T lymphotropic virus) infected, cytokine independent, human T cell line developed from cells isolated from a patient diagnosed with mycosis fungiodes. MOLT4 and CCRF-CEM are cytokine independent T cell lines derived from the peripheral blood of two different patients with acute lymphoblastic leukemia. These three cell lines were obtained from the ATCC.

MT-1 is an HTLV1 infected cytokine independent human T cell line (Tsudo M, *et al.* "The p75 peptide is the receptor for interleukin 2 expressed on large granular lymphocytes and is responsible for the interleukin 2 activation of these cells" *Proc Natl*

Acad Sci USA 84:5394-5398, 1987). Kit 225 is an IL-2 dependent T cell line derived from a patient with T cell chronic lymphocytic leukemia (Hori, T., *et al.* "Establishment of an interleukin 2-dependent human T cell line from a patient with T cell chronic lymphocytic leukemia who is not infected with human T cell leukemia/lymphoma virus" *Blood* 70:1069-1072, 1987). Additional cytokine independent clones used were isolated from the IL-2 dependent Kit 225 parent. YT is a human natural killer (NK) like cell line derived from a patient with acute lymphoblastic lymphoma (Yodoi, J., *et al.*, "TCGF (IL 2)-receptor inducing factor(s). I. Regulation of IL 2 receptor on a natural killer-like cell line (YT cells)" *J Immunol* 134:1623-1630, 1985). Dukes cultured T cells (CTC) and Jean-Gilles CTC are HTLV1 infected and IL-2 dependent T cell lines developed from the cells of patients with adult T cell leukemia (ATL). 1C9 and 5B4 are Epstein-Barr virus (EBV) transformed normal β cell lines. The MOPC 104E murine plasmacytoma line secretes a pristane-induced non-immune mouse IgM myeloma protein (Potter M., "Immunoglobulin-producing tumors and myeloma proteins of mice" *Physiol Rev.* 52:631-719, 1972).

All cell lines were cultured in RPMI 1640 media supplemented with 10% heat inactivated fetal bovine serum (Life Technologies) in 5% CO_2 at 37°C, unless otherwise indicated. Cytokine dependent T cell lines were additionally supplemented with 100 units/ml recombinant human IL-2 (Hoffmann-LaRoche). For large scale hollow fiber bioreactor production of CKG Hd5F7, the hybridoma was adapted to serum-free culture in Hybridoma SFM (Life Technologies) and was grown in a small Cellco system using a Cellmax hollow fiber cartridge (Spectrum Laboratories).

EXAMPLE 3

Detection and Identification of ILRAPs

Although an understanding of the mechanism is not necessary to use the present invention, this Example describes experiments to detect and identify ILRAPs. The multisubunit IL-2 receptor consists of the private IL-2Rα subunit as well as the shared IL-2Rβ and γc subunits. In addition, ICAM-I (molecular weight 90 kDa) and Class I MHC (molecular weight 40-45 kDa) are non-randomly associated with IL-2Rα. In an effort to search for additional IL-2R associated proteins, a series of monoclonal antibodies was produced by immunizing mice with monoclonal antibodies directed to human IL-2. The approach was based on the strategy that an antibody to the idiotype of such an anti-IL-

2 antibody might have a shape similar to IL-2 itself and thus might bind to an IL-2R associated protein. One such immunization series was performed using the monoclonal antibody 7B1 (this antibody binds to a fragment of human IL 2 encompassing amino acids nine to nineteen of the IL-2 molecule) as the antigen. The resultant monoclonal antibody producing hybridomas (*See*, Example 1) obtained from this immunization were screened to identify antibodies that reacted with the high affinity IL-2R expressing cells such as the HutT 102 cells or the IL-2Rβ and γc expressing cells which can be induced to express the IL-2Rα subunit such as the YT cells, but not with cells that do not express IL-2Rα and IL-2Rβ such as the CEM cells (Figure 1). Using this strategy, an IgMK monoclonal antibody 5F7 was isolated.

The strategy of the receptor distribution (above) and co-modulation studies (Example 5) is as follows. To investigate the relationship between ILRAP and the IL-2R, various cell lines were analyzed by FACS to determine their relative expression of each component chain of the IL-2R concomitant to their expression of ILRAP, as described above. Two such cell lines, Kit 225 and Hut 102, leukemic T cell lines positive for ILRAP as well as the αβγ chains of the IL-2R, were selected for further study to determine the effect of IL-2 on the expression of ILRAP in the presence of the high affinity IL-2R. Rapidly growing cells were cultured in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS) at 37°C for 1 hr with and without 100 units/ml recombinant human IL-2. Under such conditions in the presence of IL-2, cells bearing the high affinity IL-2R will internalize the IL-2 that has bound to this receptor. At the end of the culture period, cells were immediately washed with cold FACS buffer to terminate metabolic activity and then were stained for IL-2R and ILRAP expression by FACS analysis on ice, as described above. Duplicate cultures with and without IL-2 were maintained at 4°C during the incubation period. Relative amounts of receptor expression were always compared to a nonimmune mouse immunoglobulin (Ig) control as background. Receptor internalization was said to have occurred if a chain expressed on cells held at 4°C in the presence of cytokine could no longer be detected on the cells allowed to incubate at 37°C in the presence of cytokine.

In the receptor distribution studies above and co-modulation studies (Example 5) the reactivity of a specific antibody with a particular cell was detected by multi-parameter flow cytometry FACS (fluorescence activated cell sorter) analysis using a Becton Dickinson FACScan cell analyzer.

In general, cells were prepared for FACS analysis as follows:

a) Cells were washed 3x in Hank's balanced salt solution (BSS) and dispensed 10^6/ tube in 100 µl of FACS buffer;

b) Human gamma globulin (10 µg) dissolved in FACS buffer was added to each tube to block nonspecific binding of subsequent reagents, and the cells were incubated on ice for 15 minutes.

c) Appropriately titrated antibody was added to each tube, and the cells were incubated on ice 30 minutes to 1 hour. Following incubation, the cells were washed 2x by resuspension in 3 ml FACS buffer and then pelleted by centrifugation at 1000 x g for 5 min. If the antibody used in this step was directly conjugated with a fluorochrome, the cells were now ready for fixation as in (e).

d) If the antibody used in (c) was not fluorochrome-conjugated, antibody on the surface of the cell was detected by the addition of a second antibody that was fluorochrome tagged and that would recognize the species of animal in which the first antibody was raised as well as the specific isotype of the first antibody. After the addition of this second antibody, the cells were incubated on ice for 30 min and then washed 2x as above.

e) To preserve the integrity of the stained cells until they were analyzed and also to protect against indigenous infectious agents in the cells such as HTLV1, the cells were fixed immediately following staining. Cells were resuspended in 0.5 ml fixative and incubated on ice for 15 min. Cells were washed 1x in PBS, resuspended in 1 ml of PBS, and stored refrigerated and protected from light until analyzed. Fluorescence and light scatter data collected on cells by the FACScan were analyzed using the Becton Dickinson immunocytometry systems Cellquest software version 3.1.

FACS buffer mentioned above was a phosphate buffered saline supplemented with 3% FBS and .05% Na azide. Working fixative solution is made up of one part solution #1, one part solution #2, and two parts distilled water. Stock solution #1 was 0.5 M Na cacodylate pH 7.2, and stock solution #2 was 10% paraformaldehyde.

Flow cytometric analyses (Figure 1) show that the mature T cell line HuT 102 which expresses the high affinity IL-2R, and the NK cell line YT, which expresses the IL-2R βγc receptor chains and which can be induced to express IL-2Rα both express ILRAP while the T cell line CEM, which does not express either the IL-2Rα or β chain. is negative for ILRAP.

EXAMPLE 4

Characterization of ILRAPs

Although an understanding of the mechanism is not necessary to use the present invention, this Example describes experiments to characterize the ILRAPs. Cell surface proteins from Kit 225 cells were labeled by biotinylation of the viable cells in solution using biotinamidocaproic acid 3-sulfo-n-hydroxysuccinimide ester, a membrane impermeable reagent as follows. Coupling a biotin moicty to proteins via the reaction of primary amines with N-hydroxysulfosuccinimide esters allows for the sensitive detection of thcse proteins using enhanced chemiluminescence (ECL) with strepavidin chemiluminescent enzyme conjugates. The surface proteins on intact, viable cells can likewise be tagged using a modification of standard protein biotinylation methods which allows these surface proteins to be further studied in immunoprecipitation and SDS-PAGE analysis without the use of radioisotopes. In brief, rapidly growing cells were washed free of FBS and other contaminating proteins in the culture media using Hank's BSS and resuspended at 10^7 cells/ml in 0.1 M sodium phosphate buffer, pH 7.2 (biotinylation buffer) at 4°C. The biotinylation reagent biotinamidocaproic acid 3-sulfo-n-hydroxysuccinimide ester (BAC-SulfoNHS; Sigma Chemical Co.) incorporates an aminocaproyl "spacer" arm which can reduce stearic hindrance in binding avidin to some biotinylated compounds. BAC-SulfoNHS was freshly dissolved in DMSO at a concentration of 10 mg/ml and was mixed with the chilled cells to give a final concentration of 50 µg/ml. The biotinylation reaction was allowed to proceed on ice for 40 min and was then quenched with the addition of culture media containing FBS to react with residual biotin.

These biotinylated cells were then lysed using Triton X-100 and immunoprecipitated with 5F7 monoclonal antibody-conjugated beads. Cell lysis was performed as follows. Surface biotinylated intact cells from above were washed free of contaminating proteins in Dulbecco's PBS containing 25 mM Tris pH 7.2 to which was added 150 µg/ml N-tosyl-L-phenylalanine chloromethyl ketone (TPCK) and 2mM phenylmethylsulfonyl fluoride (PMSF; Sigma Chemical Co.). Cellular proteins were solubilized by resuspension of the washed cells at a concentration of 10^8 cells/ml in the above wash buffer to which was added 1% Triton x-100 (Pierce Chemicals) and allowing them to incubate on ice for 1 hour. Insoluble cellular membrane material was pelleted from the soluble cellular extract by centrifugation at 1500 x g for 15 minutes, and the supernatant cellular lysate was then ultracentrifuged at 100,000 x g for 40 minutes to pellet

DNA and any residual debris. Solubilized cell lysates were recovered and stored at -70°C until use.

Immunoprecipitation was performed as follows. To biochemically characterize ILRAP and study its physical association with IL-2R, immunoprecipitation and cross clearing studies were performed using solubilized lysates of surface biotinylated cells. All antibodies used for immunoprecipitation were directly coupled to rigid agarose beads (NHS-activated Sepharose 4 Fast Flow; Pharmacia Biotech) according to manufacturer instructions. All immunoprecipitation studies were performed in Tris buffered saline containing 1% Triton X-100 and protease inhibitors (lysis buffer) as described above.

In brief, cellular lysate from 10^8 cells was allowed to incubate on ice for 30 minutes with 100 µl of agarose beads coupled with MOPC 104E, a nonimmune murine IgM antibody, with frequent vortexing. Agarose beads were pelleted at 1000 x g for 5 minutes, and the supernatant lysate was removed to a fresh tube. This preclearing step was repeated for a total of 3 cycles to remove any nonspecifically binding material from the lysate. For specific immunoprecipitation, the precleared lysate was then allowed to incubate with the antibody of interest coupled to beads for 1 hour on ice with frequent vortexing. The antibody coupled beads with bound receptor isolated from the lysate were then pelleted and saved. The supernatant lysate was discarded unless it was needed for further studies such as receptor coprecipitation. The bead/receptor complex was washed 3 times in lysis buffer to remove nonspecifically bound protein and then resuspended in the appropriate 2x SDS-PAGE sample buffer (0.125 M Tris, pH 6.8, 4% SDS, 10% 2-mercatoethanol if reducing conditions are required, 20% glycerol) to elute the immunoprecipitated proteins from the coupled antibody beads. The beads were pelleted and discarded while the supernatant immunoprecipitate was removed to a fresh tube and stored at -70°C until SDS-PAGE analysis was performed.

Selected experiments were performed in which the cellular lysate was precleared not only with nonimmune IgM coupled beads as above but also with specific antibody beads in an attempt to identify protein chains seen in common by two different antibodies If such a specific preclearing step with antibody A caused the elimination of a chain normally present when antibody B was used to immunoprecipitate but not the major peptide identified by B directly, then antibody B was said to indirectly coprecipitate the chain identified by A and thus demonstrated their close association on the cell surface.

The immunoprecipitated complex detailed as described above was then electrophoresed through a 12.5% SDS-PAGE gel, according to a modification of the Laemmli protocol as previously described (Goldman, N., and Lui, T., "Biosynthesis of human C-reactive protein in cultured hepatoma cells is induced by a monocyte factor(s) other than interleukin-1" *J.Biol.Chem.* 262:2363-8, 1987) to determine its relative molecular size. In brief, immunoprecipitated proteins dissolved in SDS-PAGE sample buffer were boiled 2 minutes, and 40 µl aliquots were loaded onto a 12.5% SDS-PAGE resolving gel containing 0.17% bisacrylamide and 0.17% diallytartardiamide (DATD) as cross-linkers using a 4.5% polyacrylamide stack. Molecular weight determinations were made using concomitantly electrophoresed SEE BLUE prestained molecular weight markers (Novex).

After electrophoresis, the biotinylated proteins immunoprecipitated from cellular lysates using antibody beads and then size separated by SDS-PAGE were visualized by electrotransfer of the proteins to a membrane followed by probing with a strepavidin conjugated chemiluminescent reagent. In brief, following protein electrophoresis, the polyacrylamide gel was equilibrated in Towbin buffer (20 mM Tris, 60 mM glycine, 20% methanol) for 15 minutes and then sandwiched in a Biorad transblot cell against a wetted polyvinylidene fluoride (PVDF) membrane (Tropix, Inc.) and electrotransferred according to manufacturer's instructions. The PVDF membrane was then removed from the gel, washed, and blocked overnight; and the biotinylated proteins on the membrane were visualized using the Western-Light Plus chemiluminescent detection system (Tropix, Inc.). This system uses a strepavidin conjugated alkaline phosphatase which binds to the biotin moiety on the proteins and causes a chemiluminescent light emission in the presence of a 1,2-dioxetane substrate (CSPD) which can be captured on film. Membranes were exposed to film for varying periods of time so that all protein bands could be visualized. Biotinylated molecular weight markers were included in all experiments to document appropriate protein transfer and successful chemiluminescent detection.

The predominant band exhibits an approximate size of 32 to 34 kDa with a less intense band at 26 to 28 kDa (Fig. 2). These two polypeptides are designated IL-2R associated polypeptides or ILRAPs. Close inspection reveals a third faint band at approximately 55 kDa, the molecular weight of IL-2R. In parallel experiments using precursor ^{35}S-methionine/cysteine biosynthetically labeled cells, an additional molecule of

14 kDa was also identified in the immunoprecipitated complex. All previously reported IL-2R are of a different size (55-105 kDa).

EXAMPLE 5

ILRAPs Associate with the IL-2 Receptor

Although an understanding of the mechanism is not necessary to use the present invention, this Example describes experiments to determine the association of ILRAPs with the IL-2R. A number of observations suggest that the polypeptides identified by the monoclonal antibody 5F7 are associated with the IL-2R. First, all of the cells examined (T cell lines HuT 102, Kit 225, Jean Gilles CTC, Dukes CTC; and EBV transformed B cell lines 1C9, 5B4) which expressed IL-2Rα also expressed the target of the new monoclonal antibody. Second, when IL-2 was added to the cell line Kit 225 at 37°C, there was a major reduction in the surface expression of IL-2Rβ (data not shown) as well as in the expression of ILRAP identified by the new monoclonal antibody. However, if the cells were held at 4°C thus preventing IL-2 internalization, ILRAP expression was not effected (Fig 3). This suggests that ILRAP is associated with IL-2R and that it co-modulates with it upon IL-2 addition.

EXAMPLE 6

ILRAPs Associate Non-Randomly with the IL-2R α Subunit

Although an understanding of the mechanism is not necessary to use the present invention, this Example describes experiments to determine the nature of association of the ILRAPs with the IL-2Rα subunit. In immunoprecipitation analyses of Kit 225 cells with the 5F7 antibody, the IL-2Rα subunit identified by anti-Tac (anti-IL-2Rα) was coprecipitated with the ILRAPs. Preclearing with anti-Tac prior to performing the immunoprecipitation and the SDS PAGE analysis removed the coprecipitate of 55 kDa molecules (Fig 4).

Flow cytometric resonance energy transfer (FRET) measurements (Szollosi J, et al. Proc Natl Acad Sci USA 1987, 84: 7246-51; Damjanovich S, et al. Proc Natl Acad Sci USA 1997, 94: 13134-139.) were used to further define the assembly and mutual proximities among ILRAP, IL-2Rα, IL-2Rβ and γc. FRET using FITC and Cy3-conjugated mAbs permits the analysis of the cell surface distribution of receptor subunits in the nanometer (2-10 nm) range. FRET analysis from donor- and acceptor-

labeled β vs. α, γ vs. α, β vs. γ, ILRAP vs. α, ILRAP vs. β and ILRAP vs. γ demonstrated a close proximity of α, β and γ subunits to each other in the plasma membrane of resting cells yielding a triangular receptor assembly. In addition, FRET measurements also showed that ILRAP and IL-2Rα are non-randomly associated on the surface of T-cell lines (Fig 5).

EXAMPLE 7

ILRAP Cloning and Sequencing

Although an understanding of the mechanism is not necessary to use the present invention, this Example describes experiments designed to isolate the ILRAPs and determine the amino acid and nucleotide sequences. The following represents the method developed to isolate and purify ILRAP. Preparative reagents are generated to isolate the specific 5F7 target, ILRAP, by conjugating the 5F7 mAb to agarose beads. Furthermore, a parallel conjugate of agarose beads to a control immune globulin is accomplished. Ultracentrifuged cell lysates from the ILRAP positive human cell, Kit 225 IG3 are exposed to the control nonimmune Ig conjugated beads to remove nonspecifically binding proteins. The unbound lysate is recovered and is then exposed to the 5F7 conjugated agarose beads for a direct affinity purification. The ILRAP/5F7 antibody complex is washed with 2.5 M KCl to remove nonspecifically bound materials and the ILRAP is then eluted from the 5F7 agarose beads with 0.1 M glycine HCL in the presence of 0.5 M NaCl. The eluted material from the column is neutralized with 1 M Tris pH 8.0, dialyzed extensively to reduce the salt concentration to 150 mM and then reconcentrated. The reconcentrated material is placed on SDS-PAGE under nonreducing conditions for a final purification based on molecular size. The resulting gel reveals an intense band at 34 - 36 kDa with a 100-fold lower expression of a band at 26 - 28 kDa. This material is transferred to a membrane for electrospray tandem mass spectroscopic analysis of the amino acid sequence. This later sequence is used to generate oligonucleotide probes for the cloning of the gene encoding ILRAP.

It is evident from the foregoing that the present invention provides novel reagents and methods for use in the treatment of various medical conditions wherein the levels of IL-2 could be regulated. Additionally, it should be evident from the foregoing that the present invention provides novel reagents and methods for diagnosis and research.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in the art of molecular biology, immunology, and/or related fields are intended to be within the scope of the following Claims.

CLAIMS

What is claimed is:

1. A composition comprising an interleukin-2 receptor associated polypeptide, wherein said interleukin-2 receptor association peptide is reactive with the monoclonal antibody produced by the hybridoma PTA-82.

2. A composition comprising the interleukin-2 receptor associated polypeptide of Claim 1, wherein said interleukin-2 receptor associated polypeptide has a molecular weight of about 32,000 to 34,000 daltons.

3. A composition comprising the interleukin-2 receptor associated polypeptide of Claim 1, wherein said interleukin-2 receptor associated polypeptide has a molecular weight of about 26,000 to 28,000 daltons.

4. A composition comprising the interleukin-2 receptor associated polypeptide of Claim 1, wherein said interleukin-2 receptor associated polypeptide is expressed by cells selected from the group consisting of Kit-225 cells and HuT 102 cells.

5. A composition comprising the interleukin-2 receptor associated polypeptide of Claim 1, wherein said interleukin-2 associated polypeptide associates with the interleukin-2 Rα subunit.

6. A composition comprising the antibody produced by the hybridoma PTA-82.

7. A composition comprising the antibody of Claim 6, wherein said antibody is monoclonal.

8. A composition comprising the antibody of Claim 7, wherein said antibody is of the IgM class.

9. A method for purifying an interleukin-2 receptor associated polypeptide, wherein said method comprises contacting cells expressing interleukin-2 receptors and interleukin-2 receptor associated protein, with an anti-interleukin-2 receptor associated polypeptide antibody under conditions wherein said antibody forms a complex with said interleukin-2 receptor associated polypeptide expressed by said cells.

10. The method of Claim 9, wherein said cells expressing said interleukin-2 receptor and interleukin-2 receptor associated protein are solubilized prior to said contacting of said cells with said antibody.

11. The method of Claim 10, wherein said anti-interleukin-2 receptor associated polypeptide antibody is present on an immunoaffinity column.

12. The method of Claim 11, wherein said interleukin-2 receptor associated protein bound to said anti-ILRAP antibody is eluted from said column.

13. The method of Claim 9, wherein said cells expressing interleukin-2 and said interleukin-2 receptor associated polypeptide are selected from the group consisting of cells lines expressing interleukin-2R α and cell lines expressing interleukin β subunits.

14. The interleukin-2 receptor associated polypeptide of Claim 13, wherein said interleukin-2 receptor expressing cells are Kit 225 cells.

15. A composition comprising an interleukin-2 receptor associated polypeptide purified by the method of Claim 9.

16. A method for detecting the presence of interleukin-2 receptor associated protein in a biological sample, comprising contacting said biological sample with an antibody produced by hybridoma PTA-82 under conditions such that said antibody and said interleukin-2 receptor associated protein in said biological sample form a complex.

17. A kit for detecting the presence of interleukin-2 receptor associated polypeptide in a biological sample comprising said antibody of Claim 16.

18. A method of treating a mammal having autoimmune disease, comprising the step of administering a therapeutically effective amount of antibody produced by hybridoma No. PTA-82 to said mammal having autoimmune disease.

19. A method of treating a mammal receiving organ transplant, comprising the step of administering a therapeutically effective amount of the antibody produced by hybridoma No. PTA-82 to said mammal receiving organ transplant.

20. A method of treating a mammal receiving immunotherapy, comprising the step of administering a therapeutically effective amount of the antibody produced by hybridoma No. PTA-82 to said mammal receiving immunotherapy.

21. The method of Claim 20, wherein said mammal has a tumor.

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(54) Title: INTERLEUKIN-2 RECEPTOR ASSOCIATED POLYPEPTIDES

(57) Abstract: The present invention relates to the identification of two polypeptides which associate with the IL-2 receptor, and are immunologically recognized by the monoclonal antibody produced from the hybridoma designated by the ATCC deposit number PTA-82. These polypeptides, designated interleukin-2 receptor associated proteins or ILRAPs, have molecular weights of about 32,000 to 34,000 daltons and 26,000 to 28,000 daltons, respectively. The invention further relates to the use of the antibody of the invention for the detection of these polypeptides and for their isolation and purification from biological samples.

FIG. 1

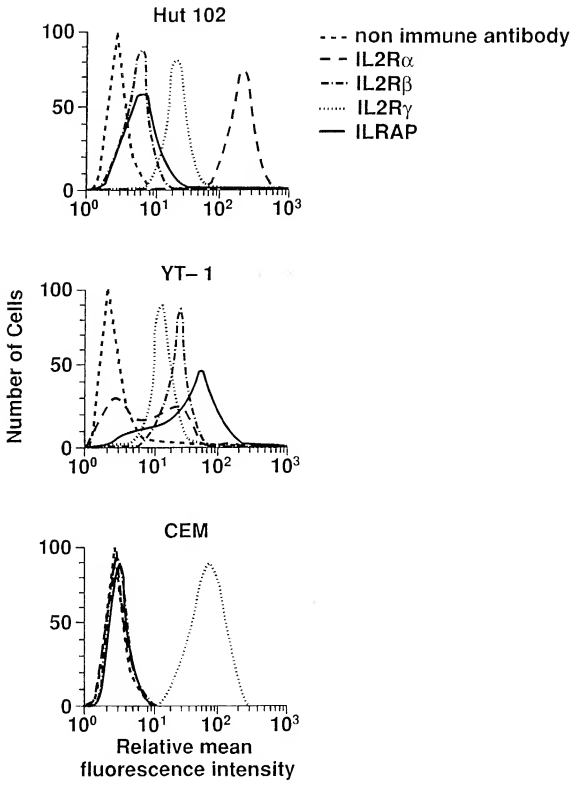
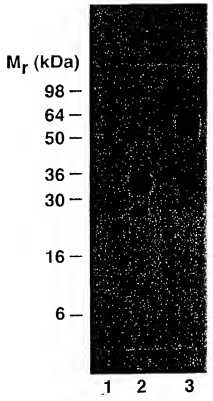

Hut 102
100
50
0
10^0 10^1 10^2 10^3
--- non immune antibody
- - IL2Rα
-·- IL2Rβ
····· IL2Rγ
— ILRAP
YT– 1
Number of Cells
100
50
0
10^0 10^1 10^2 10^3
CEM
100
50
0
10^0 10^1 10^2 10^3
Relative mean
fluorescence intensity

FIG. 2



Lane 1 MOPC 104E
Lane 2 CKG Hd 5F7
Lane 3 anti Tac

FIG. 3A

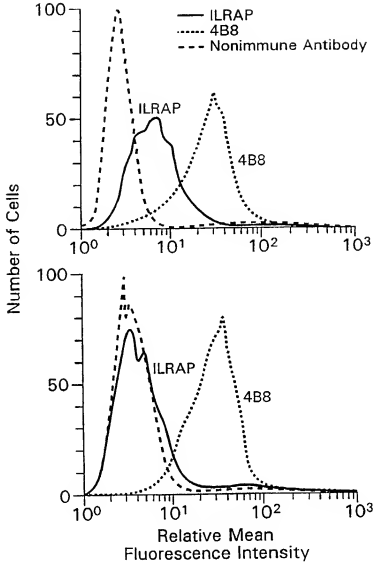

ILRAP
4B8
Nonimmune Antibody
ILRAP
4B8
ILRAP
4B8
Number of Cells
100
50
0
10^0
10^1
10^2
10^3
Relative Mean
Fluorescence Intensity

FIG. 3B

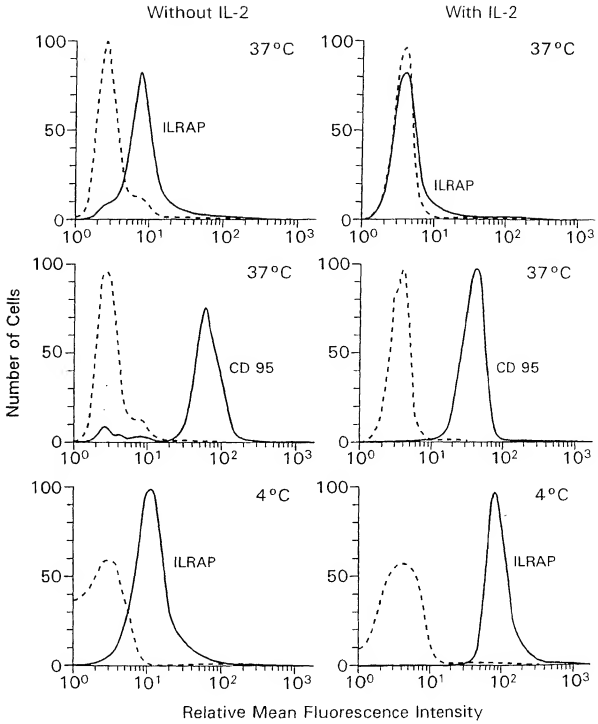
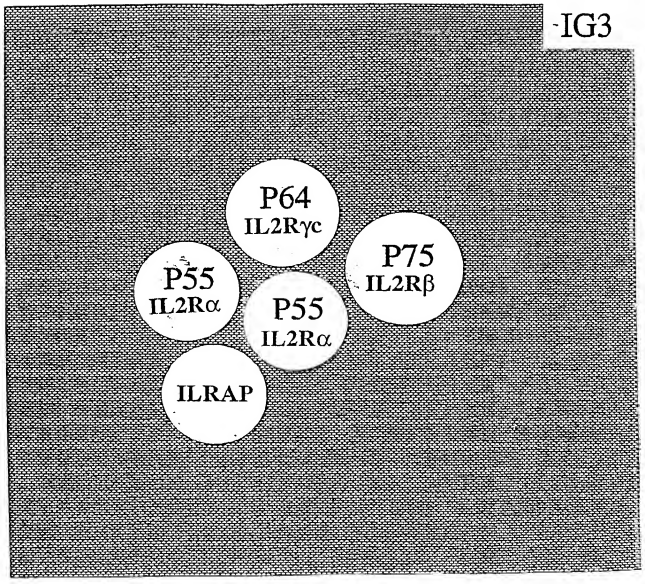

Without IL-2
With IL-2
37°C
ILRAP
CD 95
4°C
Number of Cells
Relative Mean Fluorescence Intensity
100
50
0
10^0
10^1
10^2
10^3

FIG. 4

1 2 3 4

Lane 1 MOPC 104E
Lane 2 CKG Hd 5F7
Lane 3 CKG Hd 5F7 precleared with anti Tac
Lane 4 anti Tac

FIG. 5



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Art Unit: Unassigned

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SUBSTITUTE POWER OF ATTORNEY UNDER 37 CFR 3.71

Commissioner for Patents
Washington, D.C. 20231

Sir:

The Government of the United States of America, represented by the Secretary, Department of Health and Human Services ("Assignee") is the assignee of the entire right, title, and interest in the above-identified U.S. patent application ("the application") as evidenced by an assignment recorded in the U.S. Patent and Trademark Office on August 6, 2002, at Reel 013339, Frame No. 0849. In accordance with 37 CFR 3.37(b), the undersigned certifies that the undersigned is authorized to act on behalf of the assignee, has reviewed all of the evidentiary documents accompanying or referred to in this Substitute Power of Attorney and, to the best of the undersigned's knowledge and belief, that title is in the Assignee.

The Assignee hereby revokes all previous powers of attorney and appoints the following as its attorneys to prosecute and transact all business in the U.S. Patent and Trademark Office connected with the application, including but not limited to the following: to receive all documents issued by the U.S. Patent and Trademark Office based thereon; to file continuation, continuation-in-part and divisional applications based thereon; to pay any and all fees, including maintenance fees for any resulting patent; and to file for reissues and extensions and to request reexamination of any resulting patent. All such powers are to be exercised separately or collectively.

Please recognize the National Institutes of Health, Office of Technology Transfer, as Principal Attorneys in this case: Customer Number 05318.

10089009 .080602

PATENT
Attorney Docket No.: **NIH-05111**

DECLARATION FOR PATENT APPLICATION

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated next to my name. I believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled **INTERLEUKIN-2 RECEPTOR ASSOCIATED POLYPEPTIDES**, the specification of which was filed on **03/25/2002** as Application Serial No. **10/089,009**. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PCT/US00/25963	PCT	21 September 2000	Yes
Number	*Country*	*Day/Month/Year Filed*	*Priority Claimed*

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

60/155,968	24 September 1999	Abandoned
Application Serial No.	*Filing Date*	*Patented, Pending or Abandoned*

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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In re Appln. of Goldman et al.
U.S. Patent Appln. No. 10,089,009

05318
PATENT TRADEMARK OFFICE

Please recognize Leydig, Voit, & Mayer, Ltd. as Associate Attorneys in this case: Customer Number 23460.

23460
PATENT TRADEMARK OFFICE

The assignee requests that correspondence concerning this application be directed to Leydig, Voit & Mayer, Ltd.: Customer Number 23460.

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The assignee also requests that all telephone calls be directed to Carol Larcher at (312) 616-5600.

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Date: January 31, 2003

NIH Revocation Chi. Haight (Rev. 8/13/2002)